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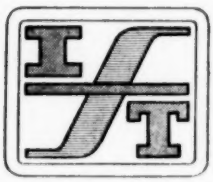

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Volume 18 JULY-AUGUST, 1953 Number 4

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PRODUCTION, USES, AND COMPOSITION OF FOODS OF PLANT ORIGIN FROM ECUADOR

HAZEL E. MUNSELL, RAÚL CASTILLO, CLEMENCIA ZURITA, AND JOSÉ M. PORTILLA

National Institute of Nutrition of Ecuador,[a] Quito

(Manuscript received December 16, 1952)

The National Institute of Nutrition of Ecuador was established with the intent of studying the composition of foods of Ecuador and the nutritional condition of the people to determine what changes should and could be recommended in dietary habits and agricultural practices so that the nutritional status of the people might improve.

As the first step in this program a food analysis laboratory was organized. The collection of samples was begun in April 1951 and analyses have been made for content of moisture, ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid.

The results obtained for 131 samples collected up to the middle of May 1952 are presented in the following pages.

METHODS OF ANALYSIS

The methods of analysis used were the same as those used by Munsell *et al.* (1949) in the study of foods of Central America.

For moisture, ether extract, crude fiber, ash, and calcium the methods of the Association of Official Agricultural Chemists *(1)* were used. Nitrogen was determined according to the procedure described by Hamilton and Simpson *(3)*. Total phosphorus was determined by the method of Lowry and Lopez *(4)* except that the solution of ascorbic used was one-tenth as concentrated as that recommended by these authors. Iron was estimated by the method of Hahn *(2)*.

The procedure for carotene was based on the methods of Moore *(5)* and Wall and Kelley *(17)* and gave values representing total carotenoid content. Concentration of

[a] The National Institute of Nutrition of Ecuador was established as a result of the efforts of Dr. José M. Portilla and with the assistance of Dr. Robert S. Harris, Professor of Biochemistry of the Massachusetts Institute of Technology. Following completion by the Government of Ecuador of a building to house the Institute an agreement was signed in July 1950, between the Government, the Pan American Sanitary Bureau (PASB), and the W. K. Kellogg Foundation (WKKF). Under the terms of this agreement the Government has provided certain facilities and salaries for employees from Ecuador, the PASB has provided technical direction of the scientific program, and the W.K.K.F. has provided funds for fellowships and for the purchase of equipment. The PASB commissioned Dr. Harris to direct the scientific program during the first years of the contract and Ecuador made him Scientific Director of the Institute. Under an appointment with the PASB (Regional Representative of the World Health Organization) Dr. Hazel E. Munsell has been holding the position of Director of the Food Analysis Laboratory and has been in residence in Quito since December 1950. Dr. Portilla holds the position of Director of the Institute.

This article is being published simultaneously in the *Bulletin of the Pan American Sanitary Bureau.*

thiamine was determined by the thiochrome technique, riboflavin was measured fluorometrically, and for niacin the microbiological procedure was used. Ascorbic acid was determined by the method of Roe and Oesterling *(15)* and the values represent total ascorbic acid in the sample at the time it was stabilized.

Natural conditions in Quito, where the laboratory is located, made it necessary to introduce some minor changes in procedures and to give particular attention in the management of certain pieces of equipment.

The altitude in the area is given as 2818 meters, atmospheric pressure ranges around 548 mm., water boils at 92°C., and atmospheric temperature seldom falls outside the range of 15 to 22°C.

The relatively low narrow-range atmospheric temperature is, in some respects, an advantage. The low atmospheric pressure on the other hand presents certain problems. Ventilation of hoods for heavy fumes is difficult. Evaporation of moisture proceeds rapidly and, although the drying oven used in the preliminary drying of slurried samples has been set at 92°C., it has been necessary to check samples carefully to avoid overdrying. In fat extraction evaporation of ether was excessive due not only to the low atmospheric pressure but also to the relatively high temperature of the tap water. This loss has been reduced somewhat by cooling the tap water before it enters the condensers by passing it through a coil immersed in an ice bath.

The effect of the low boiling temperature on crude fiber values is yet to be determined. Todd *et al. (16)* have produced evidence to show that it is definitely significant at an altitude of 6200 feet and undoubtedly it will be found to be much more significant at 9300 feet.

COLLECTION OF SAMPLES

Ecuador produces a wide variety of foods of interest to the food analyst. When the program outlined was initiated facilities were not available for collecting samples at any great distances from Quito and therefore the early samples were obtained from the various markets in the city. At that time all of the larger ones except one were outdoor markets. Foods are usually brought to these markets early in the morning and those from the immediate environs are very fresh. Those from other areas, especially the tropical fruits from the *litoral*, may have been gathered one or two days before reaching the market. Even so it was not very difficult to find representative items for sampling that were in good condition.

Many of the fruits and vegetables produced commercially in the highlands, especially in and around Ambato, were introduced either by the early Spanish settlers or later and many of these are common in European countries and in the United States. Seasonal foods seem to have adapted to the 2 seasons (winter or rainy season and summer or dry season) that prevail in production areas in this region.

Samples were usually purchased early in the morning so that the preparation of stabilized subsamples could be completed on the same day. These stabilized subsamples were prepared according to the procedures of Munsell *et al. (6)*. Determinations of ascorbic acid were usually made the day following the preparation of the subsamples and those of the other constituents as soon thereafter as possible.

PRODUCTION AND USES OF FOODS STUDIED AND DESCRIPTIONS OF SAMPLES ANALYZED

The foods listed are grouped under 5 headings: Earth Vegetables, Herbage Vegetables, Fruit Vegetables, Legumes, and Fruits. Since the common names of foods of plant origin vary from country to country and sometimes even from place to place within a country, it seemed more satisfactory to list the samples alphabetically according to the scientific, rather than the common, name. In each case the common name appears in parentheses, however, following the scientific name.

The original plan called for a botanist to collect the samples and identify the specimens of foods of plant origin.[b] Unfortunately a qualified person was not available for this position and it was necessary for the other members of the staff to assume this responsibility.

Identification of Samples Analyzed

Earth vegetables: *Allium Cepa L. (Cebolla).* The onion is an important article of diet in Ecuador and several varieties are grown, principally in the highlands and inter-Andean valleys. Onions are used in soups, in meat cookery, chopped with other vegetables, in sauces, in salads, and in many dishes where flavor is needed. The Indians make extensive use of the green tops.

For samples No. 49 and No. 50 six bunches with medium-sized bulbs and green tops, weighing 1000 g., were purchased in Quito. The bulbs and tops were washed and dried on a towel. The green tops and the outer sheaths were removed from the bulbs which were then cut in small pieces for subsampling (sample No. 49). The green tops were cut crosswise and mixed (sample No. 50). For samples No. 77 and No. 78 two bunches of onions were used. The bulbs were relatively large and the leaves green and fresh. These were prepared for subsampling in the same way as samples No. 49 and No. 50.

Arracacha xanthorrhiza Bancr. (Zanahoria blanca). This plant is a perennial herb which produces edible tapering tuberous roots resembling medium sized carrots in size and shape. The surface is deeply indented and of a dirty brown color. Produced in places where the temperature is moderate, 16-18°C., they are sold in markets throughout the country at all seasons. They are eaten boiled, fried, in soups or puree, or in croquettes prepared with egg and meat. The roots of sample No. 33 were washed thoroughly, air-dried, peeled, and cut in quarters longitudinally for subsampling.

Beta vulgaris var. *crassa Alef. (Remolacha).* Beets appear in many markets in Ecuador but are not very popular. They may be used as a cooked vegetable or in soups or salads and are consumed throughout the central provinces.

Sample No. 62 comprised 10 medium-sized, good-quality fresh roots. These were washed, dried, peeled, and then cut from top to bottom in wedge-shaped slices for subsampling.

Daucus Carota L. (Zanahoria amarilla). Carrots are grown in all highland areas throughout the year. Roots of all sizes, from 5 to 20 cm. long, appear in abundance in the markets. Carrots are used as a cooked vegetable, in soups and, cooked, in salads.

Sample No. 43 consisted of 14 roots weighing a total of 1050 g. They were washed and dried in the air, peeled, quartered, and the quarters cut crosswise in small pieces. Samples No. 57 through No. 60 were purchased at one time. Sample No. 57 comprised two roots weighing 1065 g.; sample No. 58 of three roots weighing 975 g.; sample No. 59 of six roots weighing 756 g.; and sample No. 60 of ten roots totaling 560 g. Apart from size the roots of the different samples were very similar in appearance. All were of good quality with deep yellow color. The respective lengths were roughly 19, 15, 11, and 8 cm. The roots were washed, left to dry, then cut first in half lengthwise and then in small pieces crosswise for subsampling.

Dioscorea alata L. (Papa China o Ñame). This vegetable grows in the warm lowlands and inner valleys. The root is used in soups or as a cooked vegetable. The plant is a cheap source of food since it grows freely, almost as a weed. Before cooking the roots are dried in the shade to reduce the strong flavor.

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[b] No sample was taken that could not be identified accurately. In checking fruit samples liberal use was made of the report by Popenoe *(14)* (in which the economically important fruits of Ecuador are described). Dr. Louis O. Williams of the Escuela Agrícola Panamericana, Tegucigalpa, Honduras, kindly checked several lists of names submitted to him. Mr. Wilbur Harlan of the Estación Experimental Agrícola del Ecuador y el Departamento de Agricultura de los Estados Unidos assisted in the identification of several specimens and has supplied a number of samples. Dr. Alfredo Paredes, Decano de la Facultad de Química y Farmacia de la Universidad Central willingly helped in the identification of specimens submitted to him.

Sample No. 87 consisted of 4 pieces of roots about 10 cm. long and 10 cm. in diameter. These were washed, dried, peeled, and cut in small pieces for subsampling.

Ipomoea Batatas (L.) Lam. (Camote). Sweet potatoes are produced in the low valleys. They are eaten as a cooked vegetable but, although relatively cheap, they are not highly prized as food.

Sample No. 88, *camote papa,* consisted of 14 medium-sized tubers. These were of good quality with light cream-colored flesh. They were washed thoroughly with a brush, peeled, cut in half lengthwise, and then sliced crosswise.

Sample No. 89, *camote dulce,* consisted of 15 medium-sized tubers with reddish-purple skins and light cream flesh. The flesh of this variety has a decided sweet taste. These tubers were prepared for subsampling in the same way as sample No. 88.

Oxalis tuberosa Molina (Oca). This plant is cultivated throughout the highlands at altitudes between 3000 and 3500 m. and the tuberous roots are used extensively as food by the Indians. Before being cooked they are exposed to the sun for some days to sweeten them. They are eaten as a boiled vegetable with or without sauce, or are fried in deep fat.

Four pounds of roots were purchased for subsampling. These were washed thoroughly and then divided into 2 portions. The roots of one portion, sample No. 126, were cut longitudinally and a slice taken from each for slurry C. The remainder was cut in small pieces and mixed. The second portion, sample No. 128, was exposed to the sun for 12 to 14 hours and then was subsampled as described for sample No. 126.

Raphanus sativus L. (Rábano). This vegetable is common throughout the year in the markets of Quito and elsewhere. The roots are globose with white flesh and red skin becoming whitish toward the base. It is not used by the Indians and only to a limited extent by others and then only as a raw vegetable or in salads.

Five bunches of whole plants, 20 with large roots and 37 with small ones, were purchased for sampling. These weighed a total of 800 g. and were fresh and of good quality. The leaves and roots were separated, washed thoroughly, and air dried on a towel. For one sample, No. 39, several unpeeled roots were cut in wedge-shaped slices. For another, No. 40, roots were first peeled and then sliced. The leaf blades were then used for a third sample, No. 41 (Herbage vegetables). These were broken in small pieces by hand and mixed.

Solanum tuberosum L. (Papa). The potato is native to Ecuador, Chile, and Perú. It is found in all markets in plentiful supply throughout the year. Tubers vary in length from 4 inches to 1 inch or less. The small ones are usually available in much larger quantities. Tubers of the variety sold most commonly are more or less oval with a smooth skin and yellowish flesh. They cook to an even texture, but are not mealy. Potatoes are used in many ways: in a soup *(locro)* made with water and milk, onion, cheese, *achiote,* and salt; as puree; served boiled, with or without a sauce; served fried; one dish *(llapingachos),* common in the inter-Andean region, is made from mashed boiled potatoes mixed with cheese, fat, onion, *achiote,* and salt, and fried in fat. It is said that the Indians developed one of the first methods for preserving foods by dehydration. In some regions in order to prevent loss of old potatoes through spoilage they are cut and pressed in thin slices and placed in the sun to dry. When used they are fried in fat.

Five pounds of medium-sized potatoes of the yellow-fleshed variety made up sample No. 92. For subsampling they were first washed and dried and a wedge-shaped slice cut from each and peeled for the C slurry. One half of each potato was then peeled and cut in pieces and these mixed for the other two slurries.

Ullucus tuberosus Caldas (Melloco). This perennial tuber-bearing herb is grown throughout the country at the higher elevations from 3000 to 4000 m. The tubers, which are produced underground near the base of the plant, appear in markets at all times and are relatively cheap. In some varieties the skin is yellow and in others pink. They are eaten without peeling, boiled or in soups, or sliced in salads, with lemon juice, salt, onion, and *culantro.*

Sample No. 20 consisted of small tubers with yellow skin. For subsampling they were washed thoroughly, air dried and cut in small pieces. The tubers of sample No. 122 had red skins and white flesh. After washing and drying, longitudinal slices were taken for slurry C; the remainder was sliced for the other two slurries.

Herbage vegetables: *Allium Cepa L. (Hojas de Cebolla).* For sample No. 50 see Earth Vegetables, sample No. 49. For sample No. 78 see Earth Vegetables, sample No. 77.

Brassica oleracea var. *acephala D. C. (Col verde).* Non-heading varieties of cabbage are grown only to a limited extent and most commonly in small patches by the Indian for his own consumption. The leaves are used in soups or as a cooked vegetable.

Sample No. 120 consisted of ten whole fresh plants. The leaves were dark green, fresh and tender. This material was subsampled the day following purchase. The tender leaves were removed, washed, wiped dry, the leaf blades removed from the stems by hand and broken in small pieces.

Brassica oleracea var. *botrytis L. (Coliflor).* Cauliflower, a very common vegetable in the markets of Ecuador at all times of the year, is usually very good in quality. It is used in soups, cooked as a vegetable and served with or without a white sauce, or as a cooked vegetable in salads.

Sample No. 42 consisted of 2 heads weighing 590 and 820 g. After removing the leaves and the large stem, the flower parts with the tender stems were cut in small pieces.

Brassica oleracea var. *capitata L. (Col).* Cabbage is grown extensively throughout the highlands and inter-Andean valleys. There are several varieties and some of the mature heads are of enormous size. Cabbage is eaten as a cooked vegetable, used in soups, and to a limited extent in salads. In the markets it is not uncommon to see purchases consisting of a number of separate leaves, especially from the large heads. The innermost leaves of such heads are usually treated as waste.

For samples No. 66, No. 67, and No. 68, a medium-sized head of cabbage (1500 g.) was purchased. The leaves, which were light green in color, were removed individually and placed in three small groups: outer, sample No. 66; middle, sample No. 67; and inner, sample No. 68. For subsampling the hard mid ribs were removed and the leaf blades broken in small pieces and mixed.

For samples No. 96, No. 97, and No. 98 one head of *col verde* weighing 1700 g. was used. The leaves were separated and placed in three groups: outer leaves, sample No. 96; middle leaves, sample No. 97; and inner leaves, sample No. 98. These were then subsampled the same as No. 66, No. 67, and No. 68.

Sample No. 84 consisted of two heads purchased in Ambato. The heads of this variety are long and narrow, the leaves are green and packed loosely. For subsampling the outer worm-eaten leaves were discarded, the others separated, the hard part of the stems removed, and the remainder cut in small pieces.

For sample No. 55, *col corazón*, 3 heads weighing between 800 and 1000 g. each were purchased. The leaves were light in color. After discarding injured outer leaves the remaining leaves were removed from each head in turn and placed consecutively in 6 piles. Two piles were then selected for the subsamples. The hard stems were removed from the leaves and blades then cut in small pieces and mixed.

Sample No. 125 comprised 3 small heads of so-called purple cabbage. This variety, like others, is used in salads with olive oil and lemon juice, and also as a cooked vegetable. Damaged leaves were discarded, the heads washed superficially and cut in half longitudinally. The center stem was discarded and a slice taken from each head for slurry C. The remainder was cut in small pieces and mixed.

For samples No. 69, No. 70, and No. 71 one large head of *col de seda* weighing 4800 g. was purchased. The leaves of this type of cabbage are very crisp and brittle and completely bleached. For subsampling the leaves were removed one by one and after the hard stems were removed they were made into 3 groups: outer, sample No. 69; middle, sample No. 70; and inner, sample No. 71.

One head of *col de seda* was also purchased for samples No. 95, No. 94, and No. 93. This weighed 3300 g.; the leaves were fresh and brittle and showed almost no green color. Leaves were separated as for the above samples into outer, sample No. 95; middle, sample No. 94; and inner leaves, sample No. 93.

Brassica oleracea var. *gemmifera Zenker. (Col bruscla).* This vegetable, like cabbage, is grown in the highlands and inter-Andean valleys. It is used as a cooked vegetable, in soups or in salads. Sample No. 85 consisted of a "portion" as sold in the market weighing 760 g. and made up of the little heads. The heads were of medium size, fresh, of good quality, and with a good green color. After the damaged outer leaves were removed the heads were washed, air dried, and quartered.

Brassica Rapa L. (Hojas de nabo). Turnips appear in most markets at all seasons. The leaves, which are used extensively by the poor, are eaten most commonly in soups and also in salads. The 2 samples, No. 24, and No. 35, were prepared for subsampling in the same manner. After removal of the hard stems the leaves were washed thoroughly, dried on a towel, and then broken in pieces with the hands.

Coriandrum sativum L. (Culantro). This plant, which grows to a height of 20 or 30 inches, is common throughout the highlands. The leaves have a much more pungent odor than the leaves of other Umbelliferae such as parsley and celery, and are used for flavor in soups, with meats and fish, and in vegetable salads.

The plants used for sample No. 51 were washed, air dried and the leaves and flowers removed and mixed for subsampling.

Plants for sample No. 100 were washed, the leaves and tender shoots removed and washed 5 times more, the last time with distilled water. The material was then separated into smaller pieces, dried on a towel, and subsampled.

Lactuca sativa var. *intybacea (Jacq.) Hort. (Lechuga).* Lettuce grows readily in the highland areas. It is mostly of the Iceberg variety and the heads are of good quality. It is used chiefly as a salad green. For sample No. 86 three heads weighing more than 350 g. each were purchased; the leaves were fresh and of a good green color. After removing injured leaves the heads were washed, dried, and subdivided into small pieces using a stainless steel knife.

Nasturtium officinale R. Br. (Berro). This perennial herb grows as a wild plant in most places where there is fresh water. Very cheap, it is eaten without cooking but may be used in soups. Sample No. 23 consisted of whole plants without roots. These were washed, air dried, and the leaves and terminal shoots removed and mixed for subsampling.

Petroselinum crispum (Mill.) Mansfeld (Perejil). Parsley grows in the highlands and valleys. It is used chiefly for flavor in soups, and uncooked in salads. The material for sample No. 99 consisted of fresh leaves of a good green color. After washing, the leaves and tender shoots were removed and washed 5 times more, the last time with distilled water. The material was then dried on a towel and cut in small pieces and mixed.

Raphanus sativus L. (Hojas de rábano). For sample No. 41 see Earth Vegetables, sample No. 39.

Sample No. 101 represents the wild radish that grows as a weed in cultivated fields and grasslands but at present is not used as food. Plants used for the sample were pulled in the afternoon and brought to the laboratory the following morning. After freshening for 3 hours by keeping the roots submerged in water, the leaves that were suitable for use as a cooked green were removed and thoroughly washed. The stems and hard ribs were then removed and the leaves washed once more with distilled water. After drying on a towel they were broken in pieces and mixed.

Tetragonia expansa Thunb. (Espinaca). This hardy herb is used extensively in Central and South America as a substitute for spinach. In markets of Ecuador the separate leaves are offered for sale. These are cooked as a vegetable but are generally made into a kind of sauce *(salsa)* by passing through a sieve and then seasoning with butter, onion, and spices. For sample No. 22 a "portion" was purchased. The leaves were washed and air dried on a towel; whole leaves were taken for the subsamples.

Fruit vegetables: *Cucurbita ficifolia Bouché (Zambo).* In a sense, this is a wild plant since it grows freely in gardens, or on roadside fences and at all elevations. The fruits, which are elliptical in shape with green skin marked with longitudinal white striations, appear in all markets in all seasons and are very cheap. They vary in size from small immature fruits weighing less than 1 pound to the large mature fruits which may weigh 10 pounds or more. The flesh is thick and greenish white; the numerous seeds are small and in the fruits in the markets are usually white and soft; in the fully mature fruit they are black. In general appearance this fruit resembles certain varieties of watermelon. They are eaten cooked as a vegetable, especially in soups; the flesh of mature fruits may be cooked with molasses and flavored with juice of *naranjilla* as a "dulce." In the country the Indians prepare it for cooking by removing the seeds and pulp through an opening at one end and filling the cavity with molasses. It is then set in the open fire to roast. The immature fruits are eaten

as a cold salad with onions, lime juice, and salt. Seeds from mature fruits may be toasted and eaten with salt.

Sample No. 21 consisted of 11 small very immature fruits. For subsampling they were washed, dried, peeled, the seeds removed, and the flesh cut in small pieces. Sample No. 26 consisted of 2 fruits about 30 cm. long and weighing about 4700 g. each. A longitudinal wedge was cut from each, pealed, the seeds removed and the flesh cut in small pieces for subsampling.

Cucurbita Pepo L. (Castellano, Zapallo). This plant is cultivated more or less casually since the fruit is not greatly appreciated although it is eaten in large amounts by the Indians because it is cheap. It is used in soups or for the preparation of a dulce.

Sample No. 127, consisted of one large fruit weighing about 8000 g. and which resembled closely fruits of C. maxima. For subsampling a longitudinal quarter section was cut and the seeds removed. A longitudinal slice was then cut from this, peeled, and used immediately for the C slurry. The remainder was peeled and cut in small pieces.

For sample No. 37 one fruit weighing about 8 pounds was used. The skin was green and the flesh yellowish green. For subsampling the skin and seeds were removed and the flesh cut in small pieces.

Samples No. 147 and No. 148 came from one very large squash. For sample No. 147 a longitudinal section was cut from the side that had been exposed to the sun. For sample No. 148 a similar longitudinal section was taken from the side that had rested on the ground. These were then subsampled in the same manner as sample No. 127.

Lycopersicum esculentum Mill. (Tomate extranjero). This variety of tomato is grown in the warm parts, especially near the coast. The seed is imported. The fruits are marketed throughout the year but are in greatest supply from June to October when they are reasonably priced. The fruits are oblate spherical and the surface is smooth with few if any indentations. Tomatoes are used in salads or in soups and sauces *(salsas)* for flavor.

Sample No. 32 comprised 12 good-quality ripe fruits deep red in color and weighing about 120 g. each. For subsampling they were washed and dried, sliced and cut in small pieces. The skin was included. For sample No. 61 seven good-quality, medium-size, fully ripe fruits of good red color were purchased. After washing and drying they were cut in wedge-shape slices for subsampling.

Lycopersicum esculentum Mill. (Tomate del país o Tomate riñón). These fruits are small, somewhat flattened and deeply furrowed. They are generally used for flavor in soups, sauces, and other dishes.

For sample No. 48 thirty-seven good-quality fruits weighing 845 g. were purchased. The fruits were washed, wiped dry and cut in pieces for subsampling. For sample No. 54 three pounds (30-40 fruits) were purchased. They were subsampled in the same manner as fruits of sample No. 48. Sample No. 65 consisted of 2 pounds of good-quality small red fruits. They were washed, wiped dry, and then sliced from top to bottom.

Sechium edule (Jacq.) Sw. (Achogcha). This cucurbit grows readily in many parts of the country. The *achogcha* fruits are blunt at the stem end and sharply pointed at the terminus. The skin is bright green and strongly ribbed. The flesh is greenish white and surrounds a number of small black seeds. The flesh is used as a cooked vegetable, in soups or, after the seeds are removed, may be used as a shell for chopped meat mixed with chopped eggs, *achiote*, and onions.

For sample No. 83 a portion, 625 g., of fruits was purchased. These were fresh and of good green color. The day following purchase they were prepared for subsampling by washing and drying, removing the seeds, and cutting the flesh—with skin—in small pieces.

Legumes: *Phaseolus vulgaris L. (Vainita).* String beans are eaten as a cooked vegetable with or without white sauce, in soups or, cooked, in salads. The string beans available in the markets consist generally of short, flat, somewhat rusty pods that tend to be rather tough after cooking.

A portion, 620 g., was purchased for sample No. 80; the pods were of medium size, young and of a good green color. After washing and drying, the ends were removed and the remainder cut in small pieces and mixed.

Pisum sativum L. (Alverja o Arveja). Peas are produced in the highland areas and inter-Andean valleys. The variety commonly grown has small pods and small seeds

TABLE 1

Composition of food samples from Ecuador

Number of the sample	Name of Food Sampled			Constituents Measured and Yields per 100 g.[c]												
	Spanish	Scientific	English	Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid, total
		Earth Vegetables		*g.*	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
49	Cebolla	*Allium Cepa L.*	Onion	87.5	.35	1.1	.286	.48	29.0	47.5	.82	.079	.060	.042	.37	18.5
77	Cebolla	*Allium Cepa L.*	Onion	85.7	.23	0.9	.182	.56	27.8	42.0	.73	.056	.036	.039	.42	14.7
33	Zanahoria blanca	*Arracacacha xanthorrhiza Bancr.*	.....	69.1	.06	0.7	.164	.97	20.4	69.9	.99	.194	.073	.029	3.50	31.8
62	Remolacha	*Beta vulgaris* var. *crassa Alef.*	Beet	89.0	.02	0.7	.220	.88	10.0	37.2	.11	.010	.010	.011	.28	4.0
43	Zanahoria amarilla	*Daucus Carota L.*	Carrot	86.6	.10	1.0	.108	.64	23.5	25.0	.61	11.108	.056	.033	.47	6.8
57	Zanahoria amarilla	*Daucus Carota L.*	Carrot	88.4	.23	1.0	.129	.81	37.7	40.8	.95	13.530	.053	.035	.58	7.7
58	Zanahoria amarilla	*Daucus Carota L.*	Carrot	88.3	.11	0.9	.136	.80	30.2	42.4	.34	13.215	.052	.035	.89	4.3
59	Zanahoria amarilla	*Daucus Carota L.*	Carrot	88.4	.12	0.9	.110	.63	28.6	21.1	.85	13.615	.045	.038	.48	4.3
60	Zanahoria amarilla	*Daucus Carota L.*	Carrot	88.1	.13	1.0	.132	.66	34.4	25.3	.82	12.420	.052	.042	.42	4.2
87	Papa china	*Dioscorea alata L.*	Yam	72.8	.07	0.8	.196	1.08	36.7	21.9	1.00	.015	.124	.027	.46	12.0
88	Camote papa	*Ipomoea Batatas (L.) Lam.*	Sweet potato	66.7	.13	1.2	.179	.86	29.3	48.1	.90	.135	.106	.035	1.23	44.0
89	Camote	*Ipomoea Batatas (L.) Lam.*	Sweet potato	71.2	.13	1.7	.132	.95	16.8	39.9	1.01	.020	.090	.028	.76	43.0
126	Oca	*Oxalis tuberosa Molina*	.....	82.4	.03	0.5	.113	.84	5.1	39.0	.90	.020	.072	.032	.42	37.3
128	Oca seca	*Oxalis tuberosa Molina*	.....	56.6	.14	1.3	.194	1.41	7.9	68.6	1.65	.029	.097	.074	1.21	41.0
39	Rábano	*Raphanus sativus L.*	Radish	92.3	.09	0.9	.106	.57	31.0	49.0	1.22	.042	.024	.021	.23	30.5
40	Rábano	*Raphanus sativus L.*	Radish	93.0	.08	0.7	.087	.49	25.0	47.2	.97	.013	.017	.018	.19	24.8
92	Papa	*Solanum tuberosum L.*	Potato	77.2	.03	0.3	.509	1.29	3.5	50.8	.96	.029	.156	.023	5.12	15.7
20	Melloco	*Ullucus tuberosus Caldas*	.....	87.9	.05	0.4	.190	.45	3.1	33.1	.89	.000	.055	.032	.26	20.4
122	Melloco	*Ullucus tuberosus Caldas*	.....	87.5	.03	0.3	.137	.63	3.7	49.0	.77	.016	.043	.028	.95	25.4
		Herbage Vegetables														
50	Hojas de cebolla	*Allium Cepa L.*	Onion tops	92.4	.50	1.3	.280	.66	50.6	42.2	2.15	2.086	.076	.094	.63	32.1
78	Hojas de cebolla	*Allium Cepa L.*	Onion tops	90.7	.52	1.3	.306	.88	60.2	55.8	5.71	2.698	.068	.109	.61	51.0
120	Col verde	*Brassica oleracea* var. *acephala D. C.*	Cabbage	87.4	.49	1.4	.676	1.58	203.7	92.9	1.94	4.807	.168	.207	1.55	220.0
42	Coliflor	*Brassica oleracea* var. *botrytis (L.)*	Cauliflower	89.5	.14	1.1	.470	.87	24.4	63.6	1.55	.038	.104	.087	.56	103.7
66	Col, hojas exteriores	*Brassica oleracea* var. *capitata L.*	Cabbage, outside leaves	91.5	.06	0.7	.213	.46	21.4	33.1	.46	.065	.077	.038	.31	39.8
67	Col, hojas intermedias	*Brassica oleracea* var. *capitata L.*	Cabbage, middle leaves	91.6	.07	0.8	.166	.40	14.0	29.9	.42	.161	.071	.031	.23	41.5
68	Col, hojas interiores	*Brassica oleracea* var. *capitata L.*	Cabbage, inner leaves	91.4	.09	0.9	.141	.42	14.5	31.8	.37	.127	.091	.019	.25	43.5
96	Col, hojas exteriores	*Brassica oleracea* var. *capitata L.*	Cabbage, outside leaves	92.9	.09	0.8	.187	.48	75.3	30.6	3.12	.131	.049	.035	.22	29.4
97	Col, hojas intermedias	*Brassica oleracea* var. *capitata L.*	Cabbage, middle leaves	92.4	.07	0.8	.199	.49	24.1	32.5	.52	.070	.057	.026	.18	24.8
98	Col, hojas interiores	*Brassica oleracea* var. *capitata L.*	Cabbage, inner leaves	91.8	.07	0.7	.270	.54	21.9	38.1	.57	.013	.063	.032	.24	28.6
84	Col chaucha	*Brassica oleracea* var. *capitata L.*	Cabbage	92.3	.24	0.8	.350	.64	35.7	44.7	.92	.389	.105	.056	1.04	61.0

[c] Credit for many of the results reported is hereby extended to Dr. Hernan Miño who worked for one year as a member of the staff of the Bromatological Laboratory.

TABLE 1 (Continued)

Number of the sample	Name of Food Sampled			Constituents Measured and Yields per 100 g.[c]												
	Spanish	Scientific	English	Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid, total
				g.	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
55	Col Corazón	*Brassica oleracea* var. *capitata L.*	Cabbage	89.5	.19	1.2	.458	.70	41.6	51.9	.31	.584	.119	.075	.46	115.5
125	Col morada	*Brassica oleracea* var. *capitata L.*	Cabbage	90.6	.09	0.8	.289	.65	27.2	36.2	.66	.049	.066	.087	.29	68.8
69	Col de seda, hojas exteriores	*Brassica oleracea* var. *capitata L.*	Cabbage, outside leaves	93.5	.15	0.7	.164	.31	22.9	17.1	.45	.065	.055	.013	.35	31.3
70	Col de seda, hojas intermedias	*Brassica oleracea* var. *capitata L.*	Cabbage, middle leaves	93.0	.12	0.7	.184	.31	24.3	17.5	.45	.065	.050	.018	.29	26.1
71	Col de seda, hojas interiores	*Brassica oleracea* var. *capitata L.*	Cabbage, inner leaves	92.1	.13	0.7	.255	.33	18.8	23.3	.50	.057	.074	.027	.28	28.0
95	Col de seda, hojas exteriores	*Brassica oleracea* var. *capitata L.*	Cabbage, outside leaves	92.3	.05	0.7	.173	.36	22.8	21.6	.82	.035	.065	.025	.23	28.4
94	Col de seda, hojas intermedias	*Brassica oleracea* var. *capitata L.*	Cabbage, middle leaves	92.8	.10	0.6	.168	.40	24.9	22.0	.56	.019	.070	.028	.17	27.0
93	Col de seda, hojas interiores	*Brassica oleracea* var. *capitata L.*	Cabbage, inner leaves	92.1	.10	0.7	.233	.47	29.2	28.7	.61	.031	.094	.031	.22	26.0
85	Col Brusela	*Brassica oleracea* var. *gemmifera Zenker*	Brussels sprouts	88.3	.27	1.5	.104	1.13	53.1	76.8	2.49	.703	.100	.131	1.04	127.5
24	Hojas de nabo	*Brassica Rapa L.*	Turnip leaves	89.0	.49	1.0	.659	1.26	250.4	56.8	1.98	5.520	.173	.455	.98	136.6
35	Hojas de nabo silvestre	*Brassica Rapa L.*	Turnip leaves	90.5	.23	0.5	.143	1.25	177.0	61.1	3.57	5.089	.124	.278	1.19	95.7
51	Culantro	*Coriandrum sativum L.*	Coriander	84.8	.91	1.8	.855	1.67	149.5	95.2	4.64	7.235	.238	.378	2.32	93.3
100	Culantro	*Coriandrum sativum L.*	Coriander	88.7	.55	1.1	.578	1.31	184.5	70.8	3.10	6.743	.225	.342	2.03	114.2
86	Lechuga	*Lactuca sativa* var. *intybacea (Jacq.) Hort.*	Lettuce	96.7	.24	0.5	.558	.28	17.1	20.9	.52	.155	.044	.028	.22	3.1
23	Berro	*Nasturtium officinale R. Br.*	Watercress	89.8	.50	0.7	.695	1.26	247.2	74.5	1.49	6.841	.197	.272	1.38	153.3
99	Perejil	*Petroselinum crispum (Mill.) Mansfeld*	Parsley	82.7	.62	1.7	.796	2.05	202.6	79.4	7.23	5.972	.208	.633	1.43	181.8
41	Hojas de rábano	*Raphanus sativus L.*	Radish Leaves	82.6	.68	1.3	.435	1.42	257.5	43.4	3.37	3.506	.117	.229	.54	110.0
101	Hojas de rábano silvestre	*Raphanus sativus L.*	Radish Leaves	88.5	.34	1.3	.465	1.06	220.2	46.2	2.33	4.020	.157	.293	.71	134.2
22	Espinaca	*Tetragonia expansa Thunb.*	New Zealand Ice plant	92.6	.33	0.6	.353	1.17	58.2	28.7	3.49	3.214	.048	.157	.64	34.8
		Fruit Vegetables														
21	Zambo	*Cucurbita ficifolia Bouché*	Squash	92.7	.09	0.3	.134	.42	12.4	41.4	.22	.000	.057	.039	.68	46.0
26	Zambo	*Cucurbita ficifolia Bouché*	Squash	94.5	.06	0.5	.056	.23	24.5	13.0	.28	.036	.021	.012	.26	18.3
127	Castellano	*Cucurbita Pepo L.*	Squash	90.4	.06	0.8	.066	.76	12.1	13.0	.62	.268	.028	.019	.43	18.6
37	Zapallo	*Cucurbita Pepo L.*	Squash	89.6	.08	0.5	.087	.39	16.3	28.2	.40	.404	.041	.029	.38	29.1
147	Zapallo	*Cucurbita Pepo L.*	Squash	90.2	.06	0.8	.094	.52	13.9	23.7	.38	1.089	.028	.017	.39	13.3
148	Zapallo	*Cucurbita Pepo L.*	Squash	88.5	.03	0.8	.115	.64	12.1	25.9	.42	1.524	.031	.022	.34	11.9
32	Tomate extranjero	*Lycopersicum esculentum Mill.*	Tomato	93.4	1.26	0.7	.144	.42	10.7	24.5	.56	.767	.090	.025	.74	28.0
61	Tomate extranjero	*Lycopersicum esculentum Mill.*	Tomato	93.6	1.00	0.8	.097	.49	6.4	28.1	.20	.550	.044	.027	.68	24.6
48	Tomate del país	*Lycopersicum esculentum Mill.*	Tomato	91.3	.63	1.2	.188	.51	6.5	31.4	.72	1.176	.077	.046	.90	38.1
54	Tomate del país	*Lycopersicum esculentum Mill.*	Tomato	91.0	.54	1.2	.209	.53	9.0	27.2	.72	1.314	.098	.060	.87	37.9
65	Tomate del país	*Lycopersicum esculentum Mill.*	Tomato	89.9	.54	1.8	.209	.66	9.9	36.5	.81	1.447	.096	.050	.91	45.1
83	Achogcha	*Sechium edule (Jacq.) Sw.*	.....	95.1	.47	0.7	.095	.60	14.3	34.2	.54	.271	.029	.035	1.10	11.5

TABLE 1 (Continued)

Number of the sample	Name of Food Sampled			Constituents Measured and Yields per 100 g.[c]												
	Spanish	Scientific	English	Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid, total
				g.	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		Legumes														
80	Vainita	*Phaseolus vulgaris L.*	String bean	91.6	.23	1.3	.309	.71	47.1	51.2	1.06	.652	.057	.129	.83	16.0
81	Arveja	*Pisum sativum L.*	Peas	72.2	.79	2.5	1.280	.75	22.0	95.6	2.55	.499	.521	.110	3.27	35.0
82	Haba	*Vicia Faba L.*	Broad bean	64.3	.13	0.6	1.971	1.10	27.0	193.3	2.96	.230	.284	.238	2.16	36.8
		Fruits														
10	Piña	*Ananas comosus (L.) Merrill*	Pineapple	88.8	.14	0.4	.065	.21	16.1	7.2	.39	.039	.049	.014	.15	27.0
56	Piña	*Ananas comosus (L.) Merrill*	Pineapple	91.2	.15	0.3	.064	.28	16.0	9.4	.34	.033	.068	.027	.13	42.5
76	Piña	*Ananas comosus (L.) Merrill*	Pineapple	89.3	.16	0.6	.054	.26	6.2	6.6	.32	.010	.048	.011	.23	58.0
1	Chirimoya	*Annona Cherimola Mill.*	Chirimoya	74.6	.45	1.5	.222	.61	21.7	30.2	.80	.000	.117	.112	1.02	16.8
112	Guanábana	*Annona muricata L.*	Soursop	83.1	.17	1.6	.138	1.06	41.4	25.9	.41	.087	.041	.053	.76	34.7
17	Chigualcán	*Carica candamarcensis Hook. f.*	.....	83.1	3.36	2.9	.505	.96	49.4	78.7	.86	.013	.111	.045	1.22	22.0
18	Chigualcán	*Carica candamarcensis Hook. f.*	.....	92.5	.23	1.2	.148	.59	13.5	16.1	.69	.068	.015	.038	1.30	47.0
13	Papaya	*Carica Papaya L.*	Papaya	90.3	.16	0.5	.085	.31	16.9	10.5	.51	.136	.021	.024	.25	54.5
52	Papaya	*Carica Papaya L.*	Papaya	90.5	.11	0.7	.081	.40	28.1	16.8	.47	.219	.037	.036	.36	62.8
118	Babaco	*Carica pentagona Heilb.*	.....	93.3	.09	0.5	.124	.57	14.1	14.0	.33	.049	.020	.022	.81	24.5
30	Limón sutil	*Citrus aurantifolia (Christmann) Swingle*	Lime	88.7	.04	0.1	.070	.25	17.1	17.3	.24	.015	.019	.016	.14	37.0
19	Lima dulce	*Citrus Limetta Risso*	Sweet Lime	91.3	.11	0.3	.086	.29	13.0	19.7	.17	.000	.063	.017	.27	51.2
31	Limón real	*Citrus Limon (L.) Burm. f.*	Lemon	92.2	.12	0.1	.075	.33	10.4	13.8	.34	.019	.037	.012	.13	22.9
27	Toronja	*Citrus maxima (Burm.) Merrill*	Grapefruit	91.3	.10	0.2	.091	.29	16.0	16.0	.24	.018	.051	.016	.29	45.5
28	Mandarina de Patate	*Citrus reticulata Blanco*	Tangerine	88.2	.10	0.4	.093	.28	28.8	14.8	.27	.049	.069	.014	.38	52.5
29	Mandarina de Sto. Domingo	*Citrus reticulata Blanco*	Tangerine	89.2	.10	0.5	.061	.28	25.0	17.6	.24	.027	.048	.014	.31	37.0
44	Naranja	*Citrus sinensis (L.) Osbeck*	Orange	89.0	.07	0.1	.074	.38	9.6	18.2	.57	.047	.056	.012	.21	51.0
72	Naranja	*Citrus sinensis (L.) Osbeck*	Orange	87.2	.11	0.1	.072	.33	14.2	20.5	.30	.031	.053	.017	.21	72.2
46	Naranja del país	*Citrus sinensis (L.) Osbeck*	Orange	90.2	.13	0.1	.064	.33	9.5	18.9	.32	.098	.053	.013	.14	72.8
36	Manzana silvestre	*Crataegus pubescens (HBK) Steud.*	.....	72.4	.17	2.7	.108	.55	59.1	47.5	.77	1.285	.021	.026	.54	119.2
116	Membrillo	*Cydonia oblonga Mill.*	Quince	81.3	.17	1.9	.203	.44	3.9	23.5	1.16	.025	.008	.010	.22	19.8
3	Tomate dulce	*Cyphomandra betacea (Cav.) Sendt.*	Tree Tomato	....	....	....	....	.70	3.9	65.5	.94	.423	.095	.035	....	33.9
12	Tomate dulce	*Cyphomandra betacea (Cav.) Sendt.*	Tree Tomato	85.0	1.28	3.0	.445	.61	11.3	60.6	.75	.571	.137	.035	1.10	32.8
74	Tomate dulce	*Cyphomandra betacea (Cav.) Sendt.*	Tree Tomato	84.8	1.17	1.9	.409	.61	4.9	52.5	.66	.653	.133	.048	1.38	25.8
129	Níspero	*Eriobotrya japonica (Thunb.) Lindl.*	.....	86.9	.14	0.5	.045	.44	14.8	13.1	.38	.270	.017	.014	.24	8.5
114	Higo	*Ficus Carica L.*	Fig	87.3	.17	2.2	.272	.83	42.3	43.2	.91	.407	.020	.016	.44	18.6
38	Frutilla	*Fragaria chiloensis (L.) Duchesne*	Strawberry	87.4	.27	1.3	.110	.32	22.3	26.4	1.38	.036	.019	.022	.42	70.0
107	Frutilla	*Fragaria chiloensis (L.) Duchesne*	Strawberry	92.3	.20	1.2	.094	.42	24.4	26.3	1.00	.043	.021	.029	.84	82.0
7	Manzana azotada	*Malus sylvestris Mill.*	Apple	85.4	.02	0.7	.070	.20	1.4	12.2	.61	.012	.017	.017	.26	31.8
5	Manzana Emilia	*Malus sylvestris Mill.*	Apple	84.9	.12	0.8	.037	.17	2.4	11.3	.26	.122	.015	.010	.20	8.8

TABLE 1 (Concluded)

Number of the sample	Name of Food Sampled			Constituents Measured and Yields per 100 g.[a]											
	Spanish	Scientific	English	Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid, total
				g.	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
6	Manzana pasposa	*Malus sylvestris Mill.*	Apple	79.8	.10	0.8	.059	.23	4.2	16.2	.43	.000	.020	.014	.23	29.9
106	Mango	*Mangifera indica L.*	Mango	79.9	.12	0.2	.061	.44	11.8	16.8	.48	1.023	.033	.036	.59	7.8
121	Zapote	*Matisia cordata Humb.* and *Bonpl.*	.....	85.3	.10	0.5	.129	.38	18.4	28.5	.44	1.056	.031	.023	.33	9.7
73	Plátano de seda	*Musa paradisiaca* var. *sapientum L.*	Banana	69.0	.19	0.5	.217	.75	8.3	35.2	.94	.662	.037	.021	.61	31.0
4	Tuna	*Opuntia Bonplandii Pfeif.*	Tuna	81.0	1.22	4.3	.105	.33	22.2	25.1	.35	.176	.003	.014	.32	28.4
9	Granadilla	*Passiflora ligularis Juss.*	Sweet Granadilla	79.0	2.32	4.4	.415	.87	13.3	64.4	.89	.035	.000	.063	1.42	21.3
115	Uvilla	*Physalis peruviana L.*	Husk tomato	78.9	.16	4.9	.054	1.01	8.0	55.3	1.23	1.673	.101	.032	1.73	43.0
108	Capulí	*Prunus Capuli Cav.*	Black cherry	76.8	.26	0.7	.185	.82	25.1	24.4	.65	.162	.031	.028	1.14	32.8
91	Mirabel	*Prunus cerasifera myrobalana (L.) C. Schneid*	Plum	89.0	.20	0.6	.105	.44	12.2	14.7	.42	.111	.017	.027	.35	8.1
109	Durazno	*Prunus persica (L.) Sieb.* and *Zucc.*	Peach	78.4	.20	0.6	.094	.53	8.6	28.6	.74	.929	.030	.023	.36	33.8
113	Guaytambo	*Prunus persica (L.) Sieb.* and *Zucc.*	Peach	81.0	.24	0.9	.064	.48	5.8	23.6	.56	.027	.026	.025	.35	28.4
119	Guaytambo blanco	*Prunus persica (L.) Sieb.* and *Zucc.*	Peach	84.2	.11	0.9	.092	.47	7.4	21.7	1.13	.000	.022	.026	.31	16.4
102	Reina Claudia	*Prunus salicina Lindl.*	Plum	87.2	.13	0.4	.126	.32	4.8	18.0	.38	.038	.010	.023	.38	8.5
103	Claudia Rosada	*Prunus salicina Lindl.*	Plum	83.2	.18	0.4	.072	.38	8.9	20.8	.51	.085	.019	.015	.43	12.2
11	Guayaba	*Psidium Guajava L.*	Guava	85.4	.29	4.4	.118	.23	20.6	21.6	.65	.274	.023	.020	1.02	186.3
117	Guayaba	*Psidium Guajava L.*	Guava	81.3	.54	7.7	.181	.58	15.9	25.7	.45	.146	.027	.036	1.15	123.1
8	Pera chirimoya	*Pyrus communis L.*	Pear	89.2	.02	1.3	.054	.25	6.4	17.7	.45	.054	.016	.020	.34	9.7
105	Pera ciruela	*Pyrus communis L.*	Pear	81.1	.13	2.5	.066	.29	5.7	16.6	.36	.020	.016	.022	.32	12.4
104	Pera de manteca	*Pyrus communis L.*	Pear	83.0	.26	2.5	.066	.26	6.3	14.4	.35	.036	.022	.021	.52	6.8
111	Pera del país	*Pyrus communis L.*	Pear	80.3	.25	1.8	.059	.34	9.0	19.9	.56	.021	.020	.016	.38	13.1
110	Pera uvilla	*Pyrus communis L.*	Pear	83.8	.23	2.2	.050	.27	8.5	12.2	.46	.036	.017	.014	.19	6.0
15	Mora de Castilla	*Rubus glaucus Benth.*	Blackberry	83.9	.49	5.4	.228	.46	34.2	36.9	.90	.000	.008	.031	.50	19.0
79	Mora de Castilla	*Rubus glaucus Benth.*	Blackberry	85.4	.59	3.9	.209	.56	32.6	37.0	3.79	.034	.010	.022	.64	14.5
16	Mora silvestre	*Rubus urticaefolius Poir.*	Blackberry	85.8	1.56	6.4	.251	.55	40.0	39.7	2.99	.005	.009	.073	.52	36.0
25	Pepino dulce	*Solanum muricatum Ait.*	Melon pear	91.0	.04	0.4	.121	.38	7.8	18.7	.54	.266	.055	.045	.53	60.0
2	Naranjilla	*Solanum quitoense Lam.*	.....	88.4	.24	4.2	.156	.66	12.4	43.7	.64	.071	.059	.033	1.20	36.3
45	Naranjilla	*Solanum quitoense Lam.*	.....	85.8	.15	4.6	.207	.75	10.0	38.7	.60	232	.065	.053	1.66	77.7
75	Naranjilla	*Solanum quitoense Lam.*	.....	86.8	.24	3.3	.188	.61	10.3	41.4	.54	.168	.094	.035	1.76	31.2
2a	Jugo de naranjilla	*Solanum quitoense Lam.*	.....	93.1	.24	0.3	....	.65	5.9	13.1	.34	.091	.070	.042	1.19	35.6
47	Jugo de naranjilla	*Solanum quitoense Lam.*	.....	90.4	.05	0.4	.107	.62	10.4	15.4	.59	.141	.057	.047	1.80	83.7
64	Ciruela hobo	*Spondias purpurea L.*	Spanish plum	86.6	.03	0.2	.102	.47	9.6	33.2	.09	.078	.106	.014	.61	31.5
90	Ciruela hobo	*Spondias purpurea L.*	Spanish plum	78.9	.09	0.2	.174	.73	6.8	42.7	.52	.076	.033	.023	1.38	26.4
14	Mortiño	*Vaccinium floribundum HBK*	Blueberry	81.1	.77	2.5	.119	.29	20.2	15.5	1.31	.038	.005	.010	.56	9.7
34	Mortiño	*Vaccinium floribundum HBK*	Blueberry	78.1	.66	2.6	.128	.28	20.4	17.6	.52	.131	.008	.013	.78	6.6

[a] Credit for many of the results reported is hereby extended to Dr. Hernan Miño who worked for one year as a member of the staff of the Bromatological Laboratory.

with 4 to 6 seeds per pod. Peas are used as a cooked vegetable, in soups, with ground meat and, cooked, in salads. Young tender seeds may be used in salads served with oil or white sauce.

Sample No. 81 consisted of a portion of pods weighing 1100 g. These were young, fresh, of good quality and of a good green color. The peas were shelled and subsampled the day following purchase.

Vicia Faba L. (Haba). This legume is grown in the highlands and inter-Andean valleys. When fresh the seeds are used as a cooked vegetable, the hard outer skin being removed before cooking. The inner skin is discarded as they are eaten, usually out of hand. When dry they are also boiled and eaten out of hand. This is one of the foods commonly eaten by the Indian as he travels from place to place. A flour is also made from the dry roasted peeled beans which may be used in soups. A portion of fresh, green, good-quality pods was purchased for sample No. 82. The seeds were removed from the pods, peeled, divided into the 2 halves and mixed.

Fruits. The country of Ecuador produces such a variety of fruits that some are always available in the markets.

Ananas comosus (L.) Merrill (Piña). Pineapples are grown in many places from sea level up to 1800 m. Those produced in the province of Guayas, especially in the vicinity of Milagro, are famous for their flavor. The pineapple is generally eaten out of hand but may be used in marmalade, ice cream, or for *refresco.*

Sample No. 10 consisted of 2 fruits weighing between 4 and 5 pounds each. The skin of each was greenish yellow and the flesh white, very juicy and of a very delicate flavor. For preparing subsamples the fruits were cut in half longitudinally, a longitudinal wedge was taken from one half of each and peeled and sliced for the C slurry. The remaining halves were peeled, cut in small pieces, and mixed.

For sample No. 56 two fully ripe fruits weighing about 1890 g. each were used. After washing and drying they were peeled and the cores removed. The flesh was then cut in small pieces and mixed for subsampling.

Sample No. 76 comprised two small, fully ripe, yellow fruits from Baños. Fruits from this area are smaller than those from the coastal area, and, although they are of poor appearance, the flesh is very juicy and has very good flavor. After peeling the fruits were sliced, the brown spots and cores removed, and the remainder cut in small pieces.

Annona Cherimola Mill. (Chirimoya). This tree, which grows only in the valleys of the sierra, was found originally growing wild in the province of Loja and is probably indigenous to Ecuador. It is now cultivated. Fruits are in season from March to June. They may vary from 100 g. or less to more than one pound in weight and are generally eaten out of hand, or used in sherbet or ice cream. The flesh is white with numerous large black seeds and has a very delicate enticing flavor.

Sample No. 1 consisted of 3 fully ripe fruits of about 500 g. each. The flesh was removed, freed of seeds and mixed for subsampling.

Annona muricata L. (Guanábana). This tree is commonly cultivated in the coastal lowlands and in the low valleys but seldom above 1200 m. While the fruit is a common article of diet in the lowlands it appears in limited quantity in highland markets. It is eaten only as a sherbet.

Sample No. 112 comprised two fully ripe fruits, one large and one small, totaling 1875 g. After washing and drying the white flesh was freed of seeds and mixed.

Carica candamarcensis Hook. f. (Chigualcán). This tree, which is probably indigenous to northern South America and is common throughout the sierra, has a somewhat stouter trunk than the papaya. The fruits are elliptical, sharply pointed at the apex and deeply 5-sutured and are smaller than fruits of *C. Papaya* and *C. pentagona.* The seeds are included in a gelatinous pulp. When ripe the fruits are of a deep orange color. They are sold commonly in highland markets and are generally eaten cooked in conserves or as a "dulce." The sample consisted of 13 ripe fruits weighing about 175 g. each. For subsampling they were peeled and two portions made, one of the seeds with surrounding pulp, sample No. 17, and the other from the true flesh, sample No. 18.

Carica Papaya L. (Papaya). This fruit is grown in all parts of the country at elevations up to 1800 m. There are a number of excellent varieties, some of which are superior to those grown in other countries. It is generally eaten out of hand.

For sample No. 13 two fruits, about 5 pounds each, were purchased in Quito. One was fully ripe and the other slightly under-ripe. For subsampling a longitudinal wedge

was taken from each. These were peeled, the seeds removed, and the remainder cut in small pieces.

For sample No. 52 three fruits weighing between 775 and 875 g. were purchased in Quito. These were fully ripe and of good quality. They were subsampled in the same manner as those of sample No. 13.

Carica pentagona Heilbron (Babaco). This tree grows throughout the inter-Andean area and is cultivated in many places between 1800 and 2600 m. altitude. The fruits, which are often seedless, are somewhat smaller than the papaya and the tree is seldom taller than about 3 m. The fruits are strongly 5-angled with flesh about 1.5 to 2 cm. thick and are eaten only after cooking. The flesh may be cut in slices or in small pieces and mixed with orange or *naranjilla* juice. It is often eaten with *mazamorra morada.* (see *Vaccinium floribundum*).

Sample No. 118 consisted of 2 fruits weighing about 700 g. each. After washing they were peeled and the flesh sliced lengthwise and then across into small pieces.

Citrus aurantifolia (Christmann) Swingle (Limón sutil). This tree grows in the lowlands and in warm areas up to 1800 m. This is the "lemon" of tropical countries; the fruit is used for *refrescos* and for flavor in cooking. It is also used in large quantities, chiefly by the Indians, as a medicine.

Sample No. 30 consisted of 25 fruits of about 40 g. For subsampling the juice was extracted by means of a glass reamer.

Citrus limetta Risso (Lima dulce). This tree grows throughout the country at elevations up to 2000-2500 m. In appearance the fruit resembles the lemon; it is very juicy with a sweet but somewhat insipid flavor and is generally eaten out of hand. At present the number of trees is rather limited due to disease from an invading parasite "El Coma del Citrus" *(Lepidosaphes beckii; Lepidosaphes gloveri).*

For sample No. 19 twenty-four fruits were purchased. These averaged 90 g. and were fully ripe with yellowish green skins. After the outer skin, the inner white membrane and the seeds were removed, the segments were bisected and mixed for subsampling.

Citrus Limon (L.) Burm. f. (Limón real). The lemon grows in Tungurahua and Imbabura provinces and other warm valleys and on the coast, but nowhere is it of commercial importance. The fruits are larger than those of *limón sutil* but the skin is thick, the pulp is rather dry, and the flavor is less pleasing. The fruits may be used for *refrescos* or in marmalade.

For sample No. 31 twenty-five fruits, weighing about 140 g. each, were purchased in Quito. Juice only was used in the subsamples.

Citrus Maxima (Burm.) Merrill (Toronja). Grapefruit trees are found near the coast and up to 1500 m. in some of the warm inter-Andean valleys. Some good varieties are now available including fruits with pink flesh as well as white. The fruit may be eaten out of hand or used for marmalades in which both flesh and skin are used.

Sample No. 27 comprised 11 fruits weighing about 500 g. each. After removing the skin, inner membrane, and seeds the segments were cut in half and mixed for subsampling.

Citrus reticulata Blanco (Mandarina). This tree is found at various places throughout the country but is cultivated commercially only in Tungurahua Province, especially in the valley of the Patate. The fruits are small with thin skins, numerous seeds and much "rag." The fruits grown in the vicinity of Santo Domingo are relatively large. The market supply is irregular according to the season and the supply is never very large. The fruits are eaten out of hand.

Sample No. 28, *mandarina de Patate,* comprised 25 fruits of 60 g. each and with orange-yellow skins. The segments, free of membranous material and seeds, were cut in half and mixed for subsampling.

Sample No. 29, *mandarina de Sto. Domingo,* comprised 25 fruits with green skins and weighing between 100 and 125 g. each. They were prepared for subsampling as fruits of sample No. 28.

Citrus sinensis (L.) Osbeck, Naranja. The orange is an important fruit in Ecuador and is eaten extensively as a fruit out of hand. Most of the trees are seedlings although work is now being done to standardize and improve varieties. They are cultivated chiefly in the coastal lowlands and to a limited extent in some warm inter-Andean valleys, notably Guayllabamba Valley, Pichincha Province, Chota Valley, Imbabura Province, and Patate Valleys in Tungurahua Province.

The 12 fruits of sample No. 44 weighed 1760 g. After washing and drying the fruits were cut in half, the seeds removed and the juice extracted with a glass reamer.

For sample No. 72 twelve fruits weighing 1130 g. were purchased in Quito. The skins were a good yellow color but the fruits were somewhat over-ripe. The juice was extracted with a glass reamer and, after removing the seeds, mixed for subsampling.

The 18 fruits of sample No. 46 weighed 3070 g. They were subsampled the same as sample No. 44.

Crataegus pubescens (HBK) Steud. (Manzana silvestre). This tree grows wild in the provinces of Pichincha and Azuay. The fruits, in season from March to May, are globose and small, about 2.5 cm. in diameter. The flesh is whitish, rather dry and mealy, but has a pleasant flavor. The fruits may be eaten out of hand or used for marmalade or jams.

Sample No. 36 consisted of mature fruits of good quality with orange-yellow skins and yellow flesh. They were washed, quartered, and the seeds removed for subsampling.

Cydonia oblonga Mill. (Membrillo). The quince grows throughout the inter-Andean area but is cultivated commercially chiefly in Ambato. The fruits are used in marmalades, conserves, *dulces,* and similar sweets.

Sample No. 116 consisted of 8 somewhat acrid fruits with greenish-yellow skins and light yellow flesh. After washing and drying a longitudinal wedge-shaped slice was removed from each and peeled for the C slurry. One half of each fruit was then peeled and the flesh sliced and mixed.

Cyphomandra betacea (Cav.) Sendt. (Tomate dulce o tomate de arbol). This tree grows throughout the inter-Andean area at elevations of 1500 to 2800 m. and is a common tree in dooryards and gardens. The fruits are oval to elliptical in shape, 7 to 9 cm. long, and orange-red in color when ripe. The flesh contains many small seeds, is orange-yellow in color and has a flavor suggestive of tomato. It is generally cooked with sugar and served as a *dulce* or conserve, or used in a *refresco.* In the high valleys *refresco* is commonly sweetened with crude sugar *(raspadura).*

Sample No. 3 consisted of 25 fruits weighing about 60 g. each. Sample No. 12 consisted of 22 good-quality, ripe fruits weighing 65 g. each. Sample No. 74 consisted of 24 good-quality, mature, medium-sized fruits. In each case the edible flesh with seeds was mixed for subsampling.

Eriobotrya japonica (Thunb.) Lindl. (Níspero). This tree grows in the highlands and may be seen occasionally in the lowlands. The globose fruits are 2 to 3 cm. in diameter with a thin layer of flesh clinging to several more or less round seeds. When ripe they are yellow or orange and the flesh is juicy and of pleasant flavor.

Sample No. 129 consisted of 85 fruits of about 15 g. each. They were ripe and of good color. For subsampling they were washed and peeled, the flesh cut in half and a slice taken from several fruits for the C slurry. The remaining flesh, freed of seeds, was cut in small pieces.

Ficus Carica L. (Higo). The fig tree in Ecuador is very successful as a fruit-bearing tree although not grown to any extent commercially. Trees are found throughout the inter-Andean area and the fruits are used chiefly for *dulces.*

Sample No. 114 consisted of 50 medium-size, green, unripe fruits. These were washed, dried, quartered lengthwise, and then sliced lengthwise. The subsamples included skin and seeds.

Fragaria chilocnsis (L.) Duchesne (Frutilla). This plant has been cultivated at Ambato since remote times. It is the strawberry of Quito and is common in several provinces but is produced on a commercial scale only near Ambato. Fruits are produced the year around but in abundance at 3 seasons, February, August, and December. The fruit is oblong-conical and when ripe is light red with pinkish-white flesh having a sweet mild flavor. It is used as a fruit out of hand, for marmalade, jams, in ice cream, and in various other ways in desserts.

Fruits of sample No. 38 were large, red, and of excellent quality. They were washed, air dried, quartered longitudinally, and mixed for subsampling.

The 100 fruits of sample No. 107 were of medium size, fully ripe, pink in color, and of good quality. They were prepared and subsampled in the same way as sample No. 38.

Malus sylvestris Mill. (Manzana). This fruit was probably introduced into Ecuador in colonial times by Spaniards from Castilla Province. Trees may be found in highland

towns from Ibarra to Loja but the chief centers of commercial production are Ambato and Cuenca. Some of the best varieties grown commercially are the *azotada*, the *Emilia*, and the *pasposa*.

The *azotada*, which ripens in February and resembles the Red Astrakan, is a fruit of medium size, with yellowish-green skin streaked with red and has a subacid flavor. It is used chiefly as a cooking apple. Sample No. 7 consisted of 20 small (60-70 g. each) ripe, good-quality fruits. After washing, one half of each fruit was peeled and the core and seeds removed. A wedge-shaped piece was taken from each and composited for determination of vitamin C. The remainder was used in the subsamples for the determination of other nutrients.

The *Emilia*, which is highly prized as an eating apple, is one of the best apples produced in Ecuador and the price may be 4 to 5 times that charged for other varieties. The season runs from March to May. On the average the fruit is 7 to 8 cm. in diameter but may reach 12 or even 15 cm. The skin is yellowish with a pink cheek; the flesh is white, very juicy, and sweet with a pleasant flavor. Five large, good quality fruits of about 280 g. each, made up sample No. 5. These were subsampled in the same way as the azotada.

The *pasposa*, which is in season from February to March or April, is a medium sized apple (90 g.) with a brownish-green slightly rough skin. The flesh has a somewhat acid flavor. It may be peeled and eaten out of hand but is more commonly used cooked as in jams, marmalades, and apple sauce. Sample No. 6 consisted of 17 medium-size, ripe, good-quality fruits. They were subsampled in the same way as the *azotada* and *Emilia*.

Mangifera indica L. (Mango). The mango grows only in the coastlands where it is a common but highly prized fruit. It is eaten out of hand.

Sample No. 106 consisted of 15 fruits weighing about 150 g. each. The skins were yellow with some black spots. The flesh was yellow, of good flavor, and with the usual number of fibers or hairs. The fruits were washed, dried, peeled, the flesh was removed from the seed, and squeezed through coarse cloth to remove the fibers.

Matisia cordata Humb. and *Bonpl. (Zapote o Sapote)*. This tree, common in the coastlands, is cultivated up to 1200 m. The name, *Sapote* or *Zapote*, should not permit confusion with *Calocarpum mammosum*, common in Central America. Fruits of *Matisia cordata* are large, up to 12 cm. in diameter, globose with rough, brownish, thick skin, and juicy orange-yellow sweet flesh containing 2 or 3 brownish seeds.

Sample No. 121 consisted of 10 fruits weighing about 300 g. each. For subsampling they were washed, cut in quarters, the flesh removed from the skin, and the seeds expressed by hand.

Musa paradisiaca var. *sapientum L. (Plátano de seda)*. Bananas are grown throughout the country at elevations up to about 2300 m., and are cultivated extensively in the coastal lowlands, in the provinces of Guayas, Manabí, and Esmeraldas, from where they are transported by truck to the highland areas. The banana is eaten in large quantities by the Indian population as a fruit out of hand, but when green they may be eaten cooked. Of the several varieties known, fruits resembling the Gros Michel variety are produced in greatest abundance.

Sample No. 73 consisted of 4 hands. They were of medium size, of good quality, unripe, and with green skins. After peeling they were cut crosswise in thin slices for subsampling.

Opuntia Bonplandii Pfeiff. (Tuna). This is a common plant in dry places along the highways and on wasteland throughout the highlands. The fruits, which are produced in abundance, are covered with short needle-like spines. The flesh of the various species of tuna varies from yellow to greenish white and is filled with small, hard round seeds. It is eaten out of hand.

Sample No. 4 comprised 16 fruits of about 100 g. each with greenish-yellow flesh. Flesh and seeds were used in the prepared sample.

Passiflora ligularis Juss. (Granadilla). The granadilla grows throughout Ecuador and is cultivated chiefly at elevations of 2200 to 2700 m. but is a common fruit in markets in all parts of the country. The vine climbs readily over trees and buildings. The fruit is oval, 5 to 8 cm. long, with a thick brittle shell, and orange-brown on the outside. The pulp encloses many small black seeds each encased in translucent juicy flesh. The flavor is slightly acid but pleasant. It is eaten out of hand.

Sample No. 9, consisted of 25 fruits averaging 60 g. For subsampling the pulp with seeds was removed and mixed.

Physalis peruviana L. (Uvilla). This plant grows spontaneously along roadsides and on the sides of cultivated fields—in all places except the low wastelands. It may also be cultivated to a limited extent. In Ambato it is grown in fields where frutillas are also grown. When sold in the markets it is relatively cheap. It is eaten out of hand or may be cooked with sugar. A preliminary treatment with hot water removes a slightly bitter flavor.

Sample No. 115 comprised 2 pounds of the fruits purchased in the husks. After removing the husks the fruits were washed, dried, and cut in half.

Prunus Capuli Cav. (Capuli). This tree grows throughout the highlands between 1800 and 3400 m. where it is more or less wild. The fruits resemble the European cherry and the common wild black cherry found in the United States and Mexico except that they are much larger than the latter. The season runs roughly from December through April when the fruits are sold in large quantities in markets and by street vendors.

For sample No. 108 two pounds of fully ripe fruits were purchased in Quito. The skins were purplish black and the flesh was greenish. The flesh and skin were removed from the seeds and mixed.

Prunus cerasifera myrobalana (L.) C. Schneid (Mirabel). This variety of plum, originally brought in from Europe, is grown only in Ambato and more extensively than other varieties. The trees are tall and produce fruit in abundance. The season runs from December through February and the red, juicy, flavorful fruits appear in abundance in the markets.

Sample No. 19 comprised 100 fruits. These were slightly under-ripe with red skins and firm flesh. After washing and drying, the flesh—with the skin—was removed from the seed and quartered.

Prunus persica (L.) Sieb. and *Zucc. (Durazno).* The peach is one of the fruits brought to Ecuador by the Spaniards and is now cultivated in all provinces of the sierra but commercially only in Tungurahua and Azuay Provinces. The name "*durazno*" is commonly reserved for fruits of the clingstone variety while "*guaytambo*" is used for the freestone type.

Sample No. 109 comprised 15 fruits of the clingstone type. They were small, not fully ripe, with yellow to greenish skins and yellowish-pink flesh. They were washed and dried, the flesh—with skin—removed from the seed and sliced.

Sample No. 113 was made up of 10 large fruits of the free-stone type weighing about 65 g. each. The skins were light green with pinkish areas and the flesh was white. They were washed and dried and the flesh with skin was sliced.

The 18 fruits of sample No. 119 weighed about 55 g. each. The skins were greenish yellow and the flesh white. After washing and drying they were broken in half and the skin and flesh sliced with a stainless steel knife.

Prunus salicina Lindl. (Claudia). This is one of the several varieties of plums grown in Ambato. It may be called the Chabot or Bailey plum and was introduced into Ecuador about 1918. The season runs from December through February and during that time the fruit is an important item in all markets and sold extensively by street vendors.

Sample No. 102, *Reina Claudia*, comprised 20 large, good-quality, ripe fruits with dark red skins and yellow flesh. They were washed and dried and the flesh—with skin—was cut into 4 parts.

Sample No. 103, *Claudia rosada*, also comprised 20 large good-quality ripe fruits with yellowish-pink skins and yellow flesh. These were treated in the same way as sample No. 102.

Psidium Guajava L. (Guayaba). The *guayaba* is one of the more common fruits and is grown from sea level to 2300 m. elevation. The round fruit, which contains many small hard seeds, is usually yellow with flesh that may vary in color from greenish white to pink or orange red. The fruit may be eaten out of hand but is more commonly used in the preparation of marmalades made with sugar, and for *dulce de guayaba* made with crude sugar *(raspadura).*

Sample No. 11, consisted of 24 fully ripe fruits weighing about 65 g. each. The skins were greenish yellow and the flesh pink. After washing and air drying a longi-

tudinal wedge was taken from each fruit for the C slurry. The remainder was cut in small pieces and mixed.

The 25 fruits for sample No. 117 were of rather inferior quality and slightly over-ripe. The skins and flesh were yellow. After washing and drying the fruits were quartered and sliced.

Pyrus communis L. (Pera). Several varieties of pears are grown in Ecuador chiefly at Ambato and Cuenca where the season runs from January through April. Propagation is usually by suckers and identification is made accordingly since there is no record of forms introduced. As a result of cross-breeding there are many varieties difficult to identify.

Sample No. 8, *pera chirimoya*, comprised 25 fruits of about 90 g. each. The skins were greenish yellow and the flesh white and of a very pleasant flavor. For subsampling the fruits were peeled and, after the seeds and cores were removed, the remaining flesh was diced and mixed.

Sample No. 105, *pera ciruela*, consisted of 20 fruits weighing about 70 g. each. They were medium-sized, of rather poor quality, under-ripe, and the skins were mixed green and brown with a reddish blush on one side. The flesh was white with a very pleasant flavor. The fruits were washed and dried, then cut in quarters and the seeds removed. The quarters, including skin, were then sliced and mixed.

Sample No. 104, *pera de manteca*, comprised 15 fruits weighing about 75 g. each. These were not fully ripe; the skins were green and the flesh white. For subsampling they were treated the same as sample No. 105.

Sample No. 111, *pera del país*, comprised 25 ripe fruits weighing about 25 g. each. The skins were greenish yellow and the flesh white. These were also sampled in the same way as sample No. 105.

Sample No. 110, *pera uvilla*, consisted of 15 slightly under-ripe fruits weighing about 60 g. each. These fruits were large for this variety; the skins were light green and the flesh white. For subsampling the sample was treated the same as No. 105.

Rubus glaucus Benth. (Mora de Castilla). This plant was originally native in the highlands of Ecuador but has been cultivated until several horticultural varieties are known. It is a vigorous grower and may reach a height of 3 m. This fruit is produced commercially in Ambato and Ibarra and throughout Imbabura Province. In flavor the fruit resembles the raspberry and is very pleasant. It is used in jams, marmalades and, in Otavalo, in a special syrup, "*arrope de mora*," used in *refrescos* and ice cream.

Sample No. 15 consisted of very dark red, fully ripe fruits. For subsampling they were cut in half longitudinally and mixed.

Fruits of sample No. 79 were large, ripe, and in very good condition. The material was prepared for subsampling the day following that on which it was purchased. The berries were quartered and mixed.

Rubus urticaefolius Poir. (Mora silvestre). This is the common wild blackberry which grows in abundance along roadside fences and around cultivated places in Tungurahua Province. The fruits are small, oval, with soft seeds, and when ripe, have a sweet agreeable flavor. They appear in markets more frequently than *Mora de Castilla*, and, like that fruit, are used in jams, marmalades, and *refrescos*. Sample No. 16 consisted of good-quality fruits. They were hulled, cut in half, and mixed for subsampling.

Solanum muricatum Ait. (Pepino dulce). This shrubby plant grows throughout the inter-Andean area at elevations between 1200 and 2700 m. The fruits are usually long and slender with yellowish skin having longitudinal purple markings. They may be seedless or with small immature seeds. The flesh is very juicy and has a crisp, subacid flavor. The fruit is more abundant in the markets at some times than at others but is generally found the year around. It is eaten out of hand. Sample No. 25 consisted of 8 fruits weighing about 280 g. each. The flesh only was used in the subsamples.

Solanum quitoense Lam. (Naranjilla). The *naranjilla* grows throughout the sierra at altitudes between 1500 and 2600 m. but is produced on a commercial scale in the Pastaza Valley region of Tungurahua Province, especially near Baños, and to a limited extent in the eastern sections of Imbabura and Pichincha Provinces. The fruits, which are covered with coarse hairs, are spherical in shape, about 5 cm. in diameter and, when fully ripe, of a bright orange color. The skin is thick and leathery in texture. The pulp, which is greenish in color and very juicy, contains many small seeds. The

juice has a pleasant subacid flavor and is used extensively as a *refresco*, as well as in ice cream. Fruits that have ripened in the sun have a sweet refreshing flavor, but the less ripe ones may be a little sour.

Samples No. 2 and No. 2a were prepared from 50 ripe fruits. The fruits were washed and the pulp removed by hand pressure. For sample No. 2 pulp with seeds was was used; for sample No. 2a the seeds were removed by use of a sieve and the pulp only was used.

The 24 fruits used for samples No. 45 and No. 47 were fully ripe, of good quality, and completely yellow. Subsamples were prepared as described above with pulp and seeds used for sample No. 45 and pulp from which the seeds had been removed for sample No. 47.

Thirty good-quality, medium-size, mature fruits made up sample No. 75; the skins were partly yellow and partly green. They were subsampled as fruits of sample No. 2.

Spondias purpurea L. (Ciruela hobo). This tree grows in the Province of Guayas and in other coastal areas and to a limited extent in the Chota Valley and is common in hedges and fence rows. The fruits are round, from 2.5 to 5 cm. in length, with a thin tough skin varying in color from yellow to deep red. The flesh is yellow and very juicy and surrounds a hard rough seed about 1 cm. in diameter. The fruits are eaten out of hand.

For sample No. 64 five pounds were purchased in Quito. These fruits were of good quality, fully ripe, and orange-yellow in color. They were washed, dried, and the pulp expressed by hand.

Sample No. 90 consisted of 100 fully ripe fruits with yellow skins. They were washed and dried and the juice then hand-expressed.

Vaccinium floribundum, HBK (Mortiños). This fruit resembles the blueberry of the United States. It is indigenous throughout the sierra where it grows abundantly at elevations from 3000 to 3700 m. The fruit is dark blue to almost black with a whitish overcast and, except for short periods at different times of the year, is found in the markets throughout the year. It is used extensively as a fruit to be eaten out of hand, in gruels made with crude sugar, in marmalades, and in *refrescos*. It is a special ingredient in *mazamorra morada*, the dish prepared with meal of dark corn, molasses, water, spices, *mortiños* and other chopped fruits (pineapple, *chamburo*, *babaco*, *naranjilla* with skin) and served on *el día de Finados*, the day (November 2) when flowers and other offerings are taken to the cemeteries. This dish is probably more common in the sierra region than at lower altitudes.

Samples No. 14 and No. 34 were purchased in Quito. The berries of sample No. 14 were fully ripe, small, and very dark blue in color. Berries of sample No. 34 were very small but sweet. The berries were washed and dried and then whole berries used in the subsamples.

RESULTS AND DISCUSSION

The results from the analyses of the 131 samples are shown in Table 1. The grouping is according to scientific names as in the previous section. With this arrangement it is easy to find data and compare values when there is more than one sample of a food. All values relate to 100 g. of raw edible material.

Since it is not possible, in this report, to compare the results shown with all data reported for similar foods from other regions, comparisons are confined to results obtained on samples from Central America by Munsell *et al. (6, 7, 8, 9, 10, 11, 12, 13).*

Earth vegetables: *Allium Cepa L. (Cebolla).* The 2 samples were similar in composition and the vitamin C values indicate fresh material.

Arracacha xanthorrhiza Bancr. (Zanahoria blanca). This root has a good value for thiamine and, compared to most roots, is high in niacin.

Beta vulgaris var. *crassa Alef. (Remolacha).* The values for the one sample of beets are very similar to the average values for the 10 samples from Central America.

Daucus Carota L. (Zanahoria amarilla). The 5 samples of carrots were remarkably similar in composition. This is of interest when considering the differences in size of roots in samples No. 57 through No. 60. Except for carotene the values parallel those

for carrots of Central America. The carotene values reported here are probably more nearly representative.

Dioscorea alata L. (Papa china). This root shows a good value for thiamine similar to the Central American sample. Several other values are slightly lower but not to a marked degree. It is of interest that the calcium value is 10 times the average for the 2 Central American samples.

Ipomoea Batatas (L.) Lam. (Camote). The 2 samples of sweet potatoes represent the white and yellow varieties. On a similar moisture basis all values are very close to those for the Central American samples.

Oxalis tuberosa molina (Oca). The 2 samples of this plant were low in calcium but contained significant amounts of thiamine and ascorbic acid.

Raphanus sativus L. (Rábano). Differences in the composition of the 2 samples of radish are in favor of the sample with peel, although the difference is not very great for any nutrient and all values are very similar to those for the samples from Central America.

Solanum tuberosum L. (Papa). The one sample of potato gave a high value for niacin and contained important amounts of thiamine. Otherwise it was similar in composition to the 14 samples from Central America. It will be of interest if additional samples of this yellow-fleshed potato give similar high values for niacin.

Ullucus tuberosus Caldas (Melloco). From a nutritive standpoint this root vegetable does not have too much to recommend it although it contains a fair share of thiamine and ascorbic acid.

Herbage vegetables: *Allium Cepa L. (Hojas de cebolla).* The fresh green tops of onions apparently contain significant amounts of calcium, carotene, thiamine, riboflavin, and ascorbic acid, and should not be discarded.

Brassica oleracea var. *acephala D. C. (Col verde).* The results obtained from the one sample of this non-heading variety of cabbage indicate that it might be a very important food. Values shown are high for calcium, carotene, thiamine, riboflavin, and niacin, and the ascorbic acid value is very high. These values are very similar to the values shown for the sample of hojas de "Kale" from Central America.

Brassica oleracea var. *botrytis L. (Coliflor).* The one sample of cauliflower contained appreciable amounts of thiamine and gave a high value for ascorbic acid.

Brassica oleracea var. *capitata L. (Col).* Results for the outside, middle, and inner leaves of the 2 heads of green cabbage, samples No. 66, No. 67, No. 68, and No. 96, No. 97, No. 98, show no consistent gradation of values according to position of the leaves except possibly in a slightly higher content of thiamine in the inner leaves. *Col chaucha.* The leaves of the one sample of this type of cabbage gave values for thiamine, riboflavin, and ascorbic acid that are higher than the values obtained for *col. Col corazón.* This variety also contained more thiamine than the samples of *col*, and much more riboflavin, and gave a high value for ascorbic acid. *Col morada.* This variety also gave higher values for thiamine, riboflavin, and ascorbic acid than *col. Col de seda.* Values for the outside, middle, and inner leaves of the 2 heads of this type of cabbage, samples No. 69, No. 70, No. 71, and No. 95, No. 94, No. 93, also showed no gradation with position of leaves, with the exception of thiamine values which indicate a higher concentration toward the center of the head. There is some indication also of a slightly higher concentration of riboflavin toward the center. In the samples of *col* this evidence of gradation of riboflavin was probably offset by the effect of the variation in greenness of leaves.

Brassica oleracea var. *gemmifera Zenker (Col Brusela).* The one sample of this Brassica, like the samples of other species, contained a good amount of thiamine. The content of riboflavin was appreciable and the value for ascorbic acid is high.

Brassica Rapa L. (Hojas de nabo). Both samples of turnip leaves gave high values for calcium, carotene, riboflavin, and ascorbic acid, and contained good amounts of thiamine.

Coriandrum sativum L. (Culantro). With its high content of calcium, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid this plant might be very important in the diet. At present it is used only to a limited extent for flavor but it might well be used as a cooked green. Some of the values shown are higher and some lower than values for corresponding nutrients in the one sample examined from Central America.

Lactuca sativa var. *intybacea (Jacq.) Hort. (Lechuga).* Lettuce is much prized as an ingredient of salads but it should probably be admitted that, judging from values so far reported, its nutritive values are low. Values shown here are similar to those reported by many other investigators including results for the 10 samples from Central America.

Nasturtium officinale R. Br. (Berro). The one sample of watercress gave high values for calcium, carotene, thiamine, riboflavin, and ascorbic acid, and contained good amounts of niacin. All of these values are appreciably higher than the average values for corresponding nutrients from the four Central American samples.

Petroselinum crispum (Mill.) Mansfeld (Perejil). Parsley leaves, like leaves of *culantro* of the same family, gave high values for calcium, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid. The values for riboflavin and ascorbic acid were much higher than those for *culantro.* Parsley is another green that might be used more extensively than it is at present.

Raphanus sativus L. (Hojas de Rábano). However fresh the leaves may be when a bunch of radishes is purchased they are very seldom used either cooked or raw. Both samples, one consisting of leaves from the conventional "bunch" and the other of leaves from wild plants gave high values for calcium, iron, carotene, thiamine, riboflavin, and ascorbic acid. From the data shown it should be apparent that this is a food to be used, not discarded.

Tetragonia expansa Thunb. (Espinaca). Leaves from the New Zealand Iceplant are the leaves most commonly served as a green vegetable in Ecuador. The sample contained appreciable amounts of carotene and riboflavin but did not give a high value for any nutrient. The values shown are surprisingly similar to the average values from the 9 samples from Central America.

Fruit vegetables: *Cucurbita ficifolia Bouché (Zambo).* This fruit is used extensively. On the basis of the values given this might well be deplored from the nutritive standpoint. The only value of any consequence is the ascorbic acid value for sample No. 21.

Cucurbita Pepo L. (Castellano y Zapallo). The four samples of squash differed considerably in appearance but with the exception of carotene values for samples No. 147 and No. 148, they were very similar in composition. Their nutritive value also paralleled closely that of the 24 samples of Central America.

Lycopersicum esculentum Mill. (Tomate extranjero). The 2 samples of this type of tomato contained no more than a fair content of any constituent. With the exception of content of ether extract and crude fiber, they were similar in composition to the 13 samples of Central America. *Tomate del país.* Although tomatoes of this type are not very attractive in appearance and are used primarily in cooking, nutritionally they are more valuable than *tomate extranjero* since they have an appreciably higher content of nitrogen, carotene, riboflavin, niacin, and ascorbic acid.

Sechium edule (Jacq.) Sw. (Achogcha). The sample of this fruit contained a significant amount of niacin but otherwise was of fairly low nutritional value.

Legumes. *Phaseolus vulgaris L. (Vainita).* The only value obtained from the sample of string beans that might be considered significant is that for riboflavin. The overall values are very similar to the average values for the 12 samples from Central America.

Pisum sativum L. (Arveja). The content of thiamine and niacin in the one sample of fresh peas analyzed may be considered high. The composition of this sample paralleled very closely the average composition of the 6 samples from Central America.

Vicia Faba L. (Haba). The values for thiamine, riboflavin, and niacin in the sample of broad bean may be considered high. The composition as a whole is very similar to the average for the 4 samples from Central America.

Fruits: *Ananas comosus (L.) Merrill (Piña).* Two of the samples of pineapple, No. 10 and No. 56, were very similar in composition. The moisture content in each was somewhat higher than the average for the Central American samples and of the other values some were lower and some higher but in no case was the difference very large. The third sample, No. 76, differed markedly in appearance from the other two. The ascorbic acid content of this sample was slightly higher than that of the other two and the content of some other nutrients slightly lower.

Annona Cherimola Mill. (Chirimoya). This fruit contains significant amounts of thiamine, riboflavin, and niacin.

Annona muricata L. (Guanábana). The sample of soursop gave a higher value for ascorbic acid but otherwise was very similar in composition to the Central American samples. Apart from ascorbic acid the nutritive content is not important.

Carica candamarcensis Hook f. (Chigualcán). The samples of this fruit gave lower values for ascorbic acid but higher values for niacin than the two samples of Papaya. The somewhat higher content of nitrogen, calcium, and thiamine in sample No. 17 is no doubt due to the seeds included.

Carica Papaya L. (Papaya). The papaya contains a good amount of ascorbic acid but does not contain other nutrients in important amounts. The two samples contained more ascorbic acid and slightly less calcium than the sample of *papaya verde* from Central America.

Carica pentagona Heilb. (Babaco). The sample of this fruit gave values not very different from those for *Chigualcán* and *Papaya*. The niacin value was between that of the other 2 fruits and the ascorbic acid value approached the lowest value.

Citrus aurantifolia (Christmann) Swingle (Limón sutil). Like other citrus fruits this lime is, except for content of ascorbic acid, low in nutritive value.

Citrus limetto Risso (Lima dulce). The sweet lime, like the *limón sutil*, contains a good amount of ascorbic acid but otherwise is low in nutritive value.

Citrus Limón (L.) Burm. f. (Limón real). This, the so-called lemon, does not even contain good amounts of ascorbic acid.

Citrus maxima (Burm.) Merrill (Toronja). The values from the one sample of grapefruit were similar to the average values for the 5 samples from Central America and, as with those samples, only the content of ascorbic acid was significantly high.

Citrus reticulata Blanco (Mandarina). The 2 samples of tangerines gave appreciably higher values for ascorbic acid than the average for the 8 samples from Central America but in other respects they were similar in composition to those samples.

Citrus sinensis (L.) Osbeck (Naranja). The ascorbic acid content of all 3 samples of oranges was higher than what is generally considered representative for oranges. Other values were similar to those for the other citrus fruits.

Crataegus pubescens (HBK) Steud. (Manzana silvestre). The sample of this apple-like fruit, like the 2 from Central America, gave a high value for ascorbic acid and contained an appreciable amount of calcium. The carotene value was higher than the average for the Central American samples.

Cydonia oblonga Mill. (Membrillo). The values obtained from the one sample examined furnished additional evidence that the quince is not nutritionally important.

Cyphomandra betacea (Cav.) Sendt. (Tomate dulce). This fruit contains small but appreciable amounts of vitamin C and appreciable amounts of niacin, no doubt from the seeds included. The values shown are very similar to those for the one sample from Central America.

Eriobotrya japonica (Thunb.) Lindl. (Níspero). The values shown indicate that this fruit is not nutritionally important.

Ficus Carica L. (Higo). The fig is another fruit that cannot be considered important nutritionally. The values obtained for thiamine and riboflavin in the one sample examined were even lower than values shown for the three Central American samples.

Fragaria chiloensis (L.) Duchesne (Frutilla). The 2 samples of this strawberry gave relatively high values for ascorbic acid similar to those shown for the three Central American samples and by other investigators for other varieties of the fruit. In respect to other nutrients the fruit is not nutritionally important.

Malus sylvestris Mill. (Manzana). The 3 samples of apples examined differed markedly in appearance but, except for content of ascorbic acid, they were very similar in composition and similar also to the three Central American samples.

Mangifera indica L. (Mango). The one sample of mango analyzed was not as high in content of ascorbic acid as might have been anticipated on the basis of data reported by other investigators. Except for content of carotene it gave low values for all other nutrients similar to those for the thirteen samples of Central America. The carotene values may be considered significantly high.

Matisia cordata Humb. and *Bonpl. (Zapote).* This is another fruit that, except for carotene content, is low in overall nutritive value.

Musa paradisiaca var. *sapientum L. (Plátano)*. The sample of banana gave an unexpectedly high value for ascorbic acid but showed the expected low content of other nutrients similar to the numerous varieties examined from Central America.

Opuntia Bonplandii Pfeiff. (Tuna). The one sample of tuna contained a fair amount of ascorbic acid but otherwise was low in nutritive value.

Passiflora ligularis Juss. (Granadilla). The sample of sweet granadilla, like other fruits in which seeds are included in the edible portion, gave a significantly high value for niacin similar to that for the five Central American samples. The values for riboflavin and ascorbic acid were fair, but the content of other nutrients was not important.

Physalis peruviana L. (Uvilla). This sample of husk tomato gave significant values for thiamine and niacin, undoubtedly due to the seeds included in the edible portion. The values for carotene and ascorbic acid are also significantly high. In overall nutritive value this fruit resembles fruits of *Physalis aequata* and *Physalis pubescens*, samples of which were examined in the Central American study.

Prunus Capuli Cav. (Capuli). The sample of this wild cherry showed a significant content of niacin and ascorbic acid but otherwise, like the sample from Central America, was low in nutritive value.

Prunus cerasifera myrobalana (L.) C. Schneid (Mirabel). This plum contained only insignificant amounts of the constituents measured.

Prunus persica (L.) Sieb. and *Zucc. (Durazno y Guaytambo)*. The three samples of peaches differed in composition in no important respect. The greatest differences were in ascorbic acid content, with the sample of the clingstone variety, No. 109, having the highest value. Except for ascorbic acid content, which on the whole was higher, these 3 samples were similar in composition to the five samples from Central America.

Prunus (salicina Lindl.) (Claudia). These two samples of plums differed little in composition from the sample of *Prunus cerasifera myrobalana* or from the five samples of *Prunus domestica* from Central America. The overall nutritive value is low.

Psidium Guajava L. (Guayaba). The 2 samples of guava gave high values for ascorbic acid, as was to be expected, and contained appreciable amounts of niacin. The composition was very similar to the average for the 4 samples from Central America.

Pyrus communis L. (Pera). These 5 samples of pears differed greatly in size, shape, and appearance of skin but the nutritive content differed very little. The composition was also very similar to the average for the five samples from Central America.

Rubus glaucus Benth. and *Rubus urticaefolius Poir (Mora)*. The only values of any significance for the blackberries were those for calcium, niacin, and ascorbic acid and even these were low. The two varities showed no differences that can be considered specific and they differed little from the Central American varities.

Solanum muricatum Ait. (Pepino dulce). Like the 5 samples from Central America this sample of melon pear gave a moderately high value for ascorbic acid but otherwise was very low in nutritive content.

Solanum quitoense Lam. (Naranjilla). The 3 samples of fruit pulp, and the two of juice, from this fruit contained good amounts of niacin and ascorbic acid and fair amounts of thiamine. The content of these nutrients would seem to justify the extensive use made of the fruit.

Spondias purpurea L. (Ciruela hobo). This so-called Spanish plum apparently varies from sample to sample in vitamin content as evidenced by the values for thiamine, niacin, and ascorbic acid. This variation was also found among the Central American samples.

Vaccinium floribundum HBK (Mortiño). In spite of the year around availability of this fruit and its rather extensive use, it is very low in nutritive value.

In the foregoing discussions of the nutritive value of the foods studied no reference has been made to iron content although some foods gave relatively high values. Before food samples were subsampled they were washed as thoroughly as seemed reasonable according to the type of the material but even with the care taken there could be no guarantee that all dirt was removed. This was especially true of leafy products and material

with deep creases such as the *moras*. Rather than run the risk of injuring samples by too thorough scrubbing it seemed better to give them a cleaning similar to native customs, for on this basis the iron value represents the expected intake as food. Until some of these foods have been carefully studied it will not be possible to interpret the iron data in terms of internal and external iron in these plants.

One often hears the statement made in Quito, usually by those who have come to Ecuador recently, that the vegetables and fruits, especially those from highland areas, are "deficient." The explanation given is that due to the heavy rains the nutrients are leached from the soil and are not replaced since fertilizers are seldom used. On the basis of data discussed in this report and from comparisons made during the course of the analytical work of data obtained in this laboratory with those reported on similar foods by other investigators it seems permissible to state that, in respect to content of nutrients for which values are given, fruits and vegetables of Ecuador compare very favorably with the same foods grown in other regions. Unless and until there is some basis in fact there is no justification for assuming that foods from this area are "deficient."

SUMMARY

In this report are presented data on the content of moisture ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid in 131 samples of foods of plant origin collected in Ecuador between April 24, 1951 and May 18, 1952. Of the 131 samples all except 30 came from material purchased in markets in Quito. The other samples were obtained in Baños, Ambato, and Machachi. A total of 75 food items, including the majority of the more commonly used fruits and vegetables, are represented in these 131 samples.

The data are discussed on the basis of values that make a food important nutritionally. Comparisons are made with values for similar foods from Central America.

Attention is called to the fact that the data presented indicate that in content of the nutrients determined the foods of Ecuador compare very favorably with similar foods grown elsewhere.

LITERATURE CITED

1. Association of Official Agricultural Chemists, *Official and Tentative Methods of Analysis*. 1945, 6th ed., Washington, D. C.
2. Hahn, P. F. Radioactive iron procedures. Purification, electroplating and analysis. *Ind. Eng. Chem., Anal. Ed.*, **17**, 45 (1945).
3. Hamilton, L. F., and Simpson, S. G. Talbot's *Quantitative Chemical Analysis*. 1946, The Macmillan Company, New York, New York.
4. Lowry, O. H., and Lopez, J. A. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.*, **162**, 421 (1946).
5. Moore, L. A. Determination of carotene in plant material. Dicalcium phosphate as an adsorbent. *Ind. Eng. Chem., Anal. Ed.*, **12**, 726 (1940).
6. Munsell, H. E., Williams, L. O., Guild, L. P., Troescher, C. B., Nightingale, G., and Harris, R. S. Composition of Food Plants of Central America. I. Hon duras. *Food Research*, **14**, 2, 144-164 (1949).

7. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., TROESCHER, C. B., NIGHTINGALE, G., AND HARRIS, R. S. Composition of Food Plants of Central America. II. Guatemala. *Food Research*, **15**, 1, 16-33 (1950).

8. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., TROESCHER, C. B., NIGHTINGALE, G., AND HARRIS, R. S. Composition of Food Plants of Central America. III. Guatemala. *Food Research*, **15**, 1, 34-52 (1950).

9. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., TROESCHER, C. B., NIGHTINGALE, G., KELLEY, L. T., AND HARRIS, R. S. Composition of Food Plants of Central America. IV. El Salvador. *Food Research*, **15**, 4, 263-296 (1950).

10. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., TROESCHER, C. B., AND HARRIS, R. S. Composition of Food Plants of Central America. V. Nicaragua. *Food Research*, **15**, 5, 355-365 (1950).

11. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., TROESCHER, C. B., AND HARRIS, R. S. Composition of Food Plants of Central America. VI. Costa Rica. *Food Research*, **15**, 5, 379-404 (1950).

12. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., KELLEY, L. T., AND HARRIS, R. S. Composition of Food Plants of Central America. VII. Honduras. *Food Research*, **15**, 6, 421-438 (1950).

13. MUNSELL, H. E., WILLIAMS, L. O., GUILD, L. P., KELLEY, L.T., MCNALLY, A. M., AND HARRIS, R. S. Composition of Food Plants of Central America. VIII. Guatemala. *Food Research*, **15**, 6, 439-453 (1950).

14. POPENOE, W. Economic fruit-bearing plants of Ecuador. U. S. National *Herbarium*, **24**, Part 5, pp. 101-134, Plates 34-49 (1924).

15. ROE, J. H., AND OESTERLING, M. J. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2,4-dinitrophenylhydrazine method. *J. Biol. Chem.*, **152**, 511 (1944).

16. TODD, J. R., 1951. Effect of altitude on the estimation of crude fiber. *Nature*, **168**, 76 (1951).

17. WALL, M. E., AND KELLEY, E. G. Determination of pure carotene in plant tissue. A rapid chromatographic method. *Ind. Eng. Chem., Anal. Ed.*, **15**, 18 (1943).

RELATIONSHIP OF SHELL EGG QUALITY AND PERFORMANCE OF EGG WHITE IN ANGEL FOOD CAKES[a]

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Several investigators have shown that the length of storage of eggs affects the ability of the egg white to produce a stable foam. Angel food cakes have been used as a means of measuring the critical factor, albumen quality. Burke and Niles *(5)* observed that the volumes of angel food cakes prepared with fresh eggs varied throughout the year. When the volumes of the cakes were plotted against the season, they found a maximum volume in February, a leveling off during March, and then a steady decline through August. In order to be strictly fresh for the best baking qualities, eggs should not be stored at room temperature [approximately 70°F.(21°C.)] for more than 4 days, according to Barmore *(1)*. Deleterious changes appeared in the older eggs which caused the cakes to have smaller volumes than those made with the fresh eggs. Refrigeration, however, seemed to be satisfactory in maintaining egg quality up to 2 weeks. Lee *(13)* found that it is the rise in pH or the concomitant carbon dioxide loss that causes the egg white to become watery.

MacDonnell, Lineweaver, and Feeney *(15)* showed that small amounts (less than 50 p.p.m.) of reducing agents, such as thioglycol, hydrogen sulfide, and sulphur dioxide not only caused egg white to thin but also caused the yolk to simulate a deteriorated appearance. They postulated that the mechanism of the nonmicrobial deterioration of shell eggs involves reduction of the S-S bonds in mucin.

In studying methods for measuring the increased volume and stability of beaten egg white, Henry and Barbour *(10)* found a greater volume was obtained with a pH above 8.0 but an increased stability of the egg white structure was obtained below this pH. Kahlenberg *(11)* held eggs at a temperature of 86°F.(30°C.) for from 4 hours to 6 days. When tested in cakes, the eggs held for the longest storage period resulted in increased shrinkage of the cakes. The method used in blending egg white samples was found to influence cake volume by MacDonnell *et al. (14)*. They found when using homogenizing pressures of from 1500 to 4600 p.s.i. there was a definite and increasing deleterious effect on viscosity of the egg white and of the volume of angel food cakes.

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Many other factors have been found that influence the volume, the flavor, the texture and grain, and the tenderness. Barmore *(1)* showed that the proportion of dry ingredients is very important in the production of a standard angel food cake. A tough cake can be due to the addition of too much flour or too little sugar. With too much sugar, the cake will be overly tender, thus causing collapse of the cell structure. A fallen cake may also result from the lack of cream of tartar, which appears to function in the coagulation of the protein. As shown by Grewe and Child *(8)*, the omission of the acid may also cause a yellow-colored cake crumb, thick cell walls, and coarse texture.

Thelan *(19)* in discussing the basic points of angel food cake preparation brings out other factors which influence quality, such as temperature of the egg white, whipping time, type of mixer or whip, speed of beating, size of mixing bowl, quantity of batter, baking temperature, and method of addition of the ingredients.

Objective tests have been applied to determine quality of angel food cakes. Most workers have used volume measurements by the method of seed displacement as developed for small loaves by Binnington and Geddes *(4)*. Penetrometer measurements have been used by Barmore *(2)* and King, Norris, and Whiteman *(12)*. Various other tests, such as compressibility, tensile strength, and photographic comparisons for cell size *(17)* have been used.

Taste panel methods *(7)* for scoring angel food cakes have been employed by Clinger, Young, Prudent, and Winter *(6)* and Miller and Vail *(16)* in their studies of frozen eggs.

The purpose of this study is to report the effect of various storage conditions of shell eggs of known history on their performance in angel food cakes.

EXPERIMENTAL

The eggs used were produced by Single Comb White Leghorn hens fed a ration containing several pigmented ingredients which resulted in golden orange yolks. The hens were fed this ration for 50 days prior to starting these studies and were trap-nested for egg collection. At the time of gathering, each egg was marked with hen number and date. The eggs were taken immediately from the hen house to an egg room [temperature 60-65°F.(15.5-18°C.)]. Each evening the eggs were placed under the following storage conditions:

Group No.	Room temperature storage
1	Stored in winter season (January to March) for 0, 1, 2, 3, 4, 5, 6, 7 weeks at 72° ± 2°F.(21.7 ± 1°C.) and relative humidity 55-65%.
2	Stored in summer season (May to July) for 0, 1, 2, 3, 4, 5 weeks, with storage conditions the same as for Group 1.
3	Duplicate of Group 2, except that temperature and relative humidity was uncontrolled; these ranged from 70-88°F.(21.1-31.1°C.) and 26 to 48%, respectively.
Group No.	**Cold Storage**
4	Placed directly in cold storage at 32°F.(0°C.).
5	Duplicate of Group 4, except that eggs were oil treated prior to storage.
6	Stored 7 days at 55°F.(12.8°C.), then oil treated and placed in 32°F. (0°C.) storage.

At the end of 2 and 4 months, samples were removed for testing. At the end of 6 months 2 samples were removed, one set was tested immediately and the other was stored at room temperature for 2 weeks and was then tested.

The storage conditions were of 3 types common to commercial practices. The 2-week period at room temperature was selected as a probable time between the removal from storage and the time the eggs are used by the consumer.

During all storage periods, the eggs were kept in wooden cases of 30-dozen capacity. When ready to be used, the eggs were removed from storage and were kept in the laboratory overnight to equilibrate them to room temperature; they were then candled into quality grades with no tolerances. The fresh (one-day old) eggs, used as a reference, were candled at the same time and matched with stored eggs from the identical hens insofar as possible.

A sample of 8 eggs was used for each trial. Eggs were weighed, broken out on a flat glass plate, and albumen height measured. Albumens were then saved and homogenized in a laboratory hand-operated homogenizer with adjustable orifice (maximum pressure 600 p.s.i.) with the orifice adjusted to open position (minimum pressure and shear) for incorporation in angel food cake formula. The formula and procedure for the angel food cakes was that given by Slosberg, Hanson, Stewart, and Lowe *(18)*. The formula consisted of 122 g. of egg white, 125 g. sugar, 45 g. cake flour, 1.8 g. cream of tartar, and 0.6 g. salt.

The weighed egg white sample was placed in the bowl of a Kitchen Aid mixer (K-4-B) and beaten for 10 sec. at speed 10 (r.p.m. 592). Cream of tartar and salt were then added. The whipping of the white was continued until the foam reached a specific gravity of approximately 0.1. The time required was from 1 to 2 min. Four-fifths of the sugar (100 g.) was added gradually for 15 sec. while the whipping was continued on speed 8 (r.p.m. 513). The bowl was scraped with a rubber spatula and the whipping continued on same speed for 15 sec. more. The specific gravity of the meringue was measured. Finally, for a period of 15 sec., the flour-sugar blend was added gradually with the beater speed set at 1 (r.p.m. 137). The bowl was again scraped and the mixing continued until the dry ingredients were well blended, a total time of 25 to 30 sec.

One hundred twenty grams of batter were weighed into each of 2 ungreased tinned baking pans (4″ x 6″ top inside x 2.75″ deep) with waxed paper in the bottom of the pan. Cakes were then baked at 350°F.(176.7°C.) for 27 min. After baking, the cakes were inverted and cooled for at least 3 hours. The volume was then measured by seed displacement and the cakes were removed from the pans. One cake of each series was wrapped in waxed paper and then in aluminum foil and frozen for later scoring *(9)*; the first and second cakes alternately were used for the objective and subjective tests.

The objective measurement employed was the amount of penetration through a one-inch cube of cake using a Precision Penetrometer with a rod attachment under a 100-g. load. At least 2 samples were taken from the center portion of each cake, the top, sides, and bottom crust having been removed.

The cakes were given to a panel of 7 persons and evaluated by the modified paired comparison test, using a known standard cake with each group of samples tested *(7)*. The characteristics of color, flavor, texture and grain, tenderness, and general acceptability were rated on 5-point numerical scales. A rating of 4 points was given each characteristic for a known-standard fresh cake to allow for any improvement which might occur during egg storage.

RESULTS AND DISCUSSION

The candled quality and albumen index measurements of eggs stored at room temperature for 2 seasons and measurements on angel food cake made from these eggs are summarized in Table 1. Similarly, the results of refrigeration storage of eggs on these measurements are presented in Table 2. Statistical analysis of the data on candled quality, albumen index values, and angel food cake measurements is presented in Table 3. The over-all picture indicates a definite decrease in the performance of egg

white when the eggs have undergone various storage treatments. There is a progressive decrease in candled quality and albumen index as the length of storage increases. The close relationship in quality drop is indicated by the negative correlation of 0.768. Albumen index values were correlated with measurements on the angel food cakes. Correlation coefficients of 0.895 between albumen index and volume, and 0.813 between albumen index and penetrometer readings were obtained. Correlation values between albumen index and taste panel factors scored a range from + 0.667 for color to + 0.815 for tenderness. All of these correlation coefficients were significant at the 1% level.

TABLE 1

Effect of controlled and uncontrolled room temperature storage during two seasons on candled quality and albumen index of eggs and quality of angel food cake

Storage period	Candled quality of eggs[a]	Albumen index of egg white	Quality measurements on angel food cake						
			Volume, ml.	Penetrometer readings, mm.	Taste panel scores				
					Color[b]	Flavor[b]	Texture and grain[b]	Tenderness[b]	General acceptability[b]
Group 1. Season I (January to March), 72° ± 2°F.									
1 day	A	102	723	17.0	3.9	3.9	3.9	4.0	3.9
1 week	A	52	660	14.8	3.9	3.3	3.9	3.6	3.3
2 weeks	A	46	630	15.0	4.0	4.0	3.8	3.8	4.0
3 weeks	B	29	578	11.7	3.8	3.5	3.7	3.7	3.7
4 weeks	B	27	568	10.4	3.6	3.4	3.6	3.3	3.4
5 weeks	B	24	560	5.6	3.4	2.7	2.9	2.7	2.6
6 weeks	C	26	575	10.4	3.3	2.5	2.3	2.7	2.3
7 weeks	C	22	540	7.3	3.5	2.8	2.9	2.7	2.8
Group 2. Season II (May to July), 72° ± 2°F.									
1 day	A	94	700	20.0	4.1	3.8	3.9	3.9	3.9
1 week	A	52	620	18.5	3.8	3.8	3.6	3.6	3.6
2 weeks	B	29	508	9.4	3.0	1.8	2.2	2.4	2.0
3 weeks	B	27	580	11.4	3.4	2.6	2.3	2.4	2.4
4 weeks	B	28	560	9.3	3.3	2.6	3.0	2.9	2.6
5 weeks	B	22	568	10.8	2.8	2.2	1.8	2.2	1.8
Group 3. Season II (May to July), 70° to 88°F.									
1 day	A	94	700	20.0	4.1	3.8	3.9	3.9	3.9
1 week	B	47	600	14.9	4.0	3.4	3.6	3.4	3.3
2 weeks	A	32	570	14.5	3.4	3.4	2.8	3.2	3.0
3 weeks	B	27	540	8.7	3.4	2.0	2.4	2.4	2.1
4 weeks	C	23	500	8.9	2.8	1.6	1.2	2.0	1.4
5 weeks	C	20	510	9.9	3.5	2.6	2.4	2.3	2.5

[a] Numerical values were given candled quality grades for statistical analysis: Grade AA—1; Grade A—2; Grade B—3; Grade C—4.

[b] Total of five points possible.

Highly significant correlations were also obtained between the volume of angel food cake and penetrometer readings and volume of angel food cake and factors scored by the taste panel, and between all factors of the taste panel score and penetrometer readings.

There is a definite effect of the season in which eggs are produced on volume of angel food cakes (F value 12.02, significant at 1% level). Cakes

prepared with eggs produced during January and February were superior to similar cakes made with eggs produced during May and June. Burke and Niles *(5)* found that the season January to March was superior to the May to July period. The decrease in cake quality paralleled the decline in albumen index values of eggs. It was reported by Berg and Bearse *(3)* that age of hens influences broken out appearance of eggs, with older hens producing eggs of lower quality. The seasonal effect found in this study is undoubtedly partially due to increased age of hens.

TABLE 2

Effect of cold storage treatments on the physical and culinary measurements on candled quality and albumen index of eggs and quality of angel food cake

Storage period	Candled quality of eggs[f]	Albumen index of egg white	Quality measurements on angel food cake						
			Volume, ml.	Penetrometer readings, mm.	Taste panel scores				
					Color[g]	Flavor[g]	Texture and grain[g]	Tenderness[g]	General acceptability[g]
Group 4. Untreated, 32°F.									
1 day	A	102	723	17.0	3.9	3.9	3.9	4.0	3.9
2 months	A	78	723	15.3	3.7	3.7	3.5	3.5	3.7
4 months	A	74	670	13.8	4.0	3.8	4.0	3.8	3.8
6 months	A	60	650	20.4	4.3	3.9	4.0	4.1	4.0
6 months 32° plus 2 wks. 70-88°F.	B	47	513	6.7	2.6	1.6	1.6	2.0	1.6
Group 5. Oil treated, 32°F.									
1 day	A	102	723	17.0	3.9	3.9	3.9	4.0	3.9
2 months	A	92	730	19.3	4.0	4.0	3.8	4.2	3.8
4 months	A	88	728	19.6	4.0	3.8	3.8	4.0	3.8
6 months	A	89	633	19.1	4.0	3.3	3.7	3.5	3.2
6 months 32° plus 2 wks. 70-88°F.	A	81	618	17.5	4.1	2.7	3.7	3.9	2.9
Group 6. Seven days at 55°F., oil treated, 32°F.									
1 day	A	102	723	17.0	3.9	3.9	3.9	4.0	3.9
2 months	A	72	673	11.7	3.3	3.5	3.3	3.7	3.5
4 months	A	61	663	15.9	4.0	3.5	3.3	3.7	3.3
6 months	A	63	615	16.1	3.4	2.2	2.8	2.8	2.4
6 months 32° plus 2 wks. 70-88°F.	C	49	583	15.3	4.0	2.5	2.8	2.8	2.5

[f] Numerical values were given candled quality grades for statistical analysis: Grade AA—1; Grade A—2; Grade B—3; Grade C—4.

[g] Total of five points possible.

The volumes of the cakes decreased when the eggs were stored at room temperature. There was a fairly rapid decrease for the first 2 weeks, and the values tend to level off after the third week. The appearance of the cakes which had a volume greater than 650 ml. was considered satisfactory to excellent. The quality improved as the volume increased as evidenced by the even texture and grain, light crumb color, and tenderness of the product. With volumes of 600 to 650 ml., the cakes were on the border

line of quality, and the texture, tended to be compact and the cakes were more moist than was desirable. With volumes below 600 ml. the cakes were gummy, tough, very coarse in texture and grain, and gray to yellow in color. Shrinkage was most evident during the last stages of baking and after removal from the oven. Often cakes with very low volumes (around 500 ml.) would fall out of the pan when inverted during the cooling period. Some variation of volume in the range below 600 ml. was possibly due to

TABLE 3

Statistical treatment of the data

Correlation coefficients[1]	r values[b]
Albumen index and candled quality	—.768
Albumen index and albumen score	—.989
Albumen index and albumen pH	—.835
Albumen index and volume of angel food cakes	.895
Albumen index and penetrometer readings for angel food cake	.813
Albumen index and acceptability ratings for angel food cake	.775
Albumen index and angel food cake	.667
Albumen index and angel food cake flavor	.725
Albumen index and angel food cake texture and grain	.758
Albumen index and angel food cake tenderness	.815
Albumen index and angel food cake general acceptability	.754
Volume of angel food cake and penetrometer readings	.794
Volume of angel food cake and acceptability ratings	.862
Volume of angel food cake and color	.716
Volume of angel food cake and flavor	.853
Volume of angel food cake and texture and grain	.824
Volume of angel food cake and tenderness	.868
Volume of angel food cake and general acceptability	.857
Penetrometer readings and acceptability ratings	.814
Penetrometer readings and color	.808
Penetrometer readings and flavor	.745
Penetrometer readings and texture and grain	.765
Penetrometer readings and tenderness	.815
Penetrometer readings and general acceptability	.767

Analyses of variance

Source of variance	d. f.	Mean squares
Seasons	1	3724[1]
Within seasons	27	321

[b] Values are significant at the 1% level.
[1] N = 58.

uneven shrinkage from the sides of the pan, because some of the cakes tended to adhere to the sides more than others. These observations on shrinkage agree with those reported by Kahlenberg *(11)*.

The average volume of angel food cakes made from day-old eggs was over 700 ml. (723 ml. for winter eggs and 700 ml. for summer eggs). These values are somewhat higher than those reported by MacDonnell *et al. (14)* who used top quality eggs as their controls. The cake volumes obtained with week-old eggs were comparable to the control values of MacDonnell *et al. (14)*. The values obtained for cake volume indicate that the low

pressure homogenization under methods used did not seriously damage the properties of the egg white.

When the data of the penetrometer measurements were correlated with albumen index and volume of angel food cakes, the r values obtained were 0.813 and 0.794—highly significant. There seem to be some discrepancies in the data which at the present time cannot be explained. In room temperature storage at different seasons, there is a progressive decrease as the storage periods increase. When a penetration value of 10 to 11 mm. is reached, the adequacy of the equipment seems to have been decreased, since values recorded on subsequent samples show little correlation. Again in Group 5 [oil treated, 32°F.(0°C.)] there is an increase in penetration values as the storage periods increase. This may possibly be due to the high concentration of carbon dioxide in oil treated shell eggs.

With the room temperature storage treatments, there was a progressive decrease in taste panel score as the storage periods increased. However, the cold storage treatments more adequately maintained the quality of the eggs for angel food cakes. The cakes made from the untreated eggs improved with age of eggs up to 6 months and the oil treated remained practically constant for the first 4 months. Group 6 treatment [7 days at 55°F.(12.8°C.) then oiled] had a deleterious effect on the quality of the cake. The last 2 weeks of storage at room temperature caused a marked decrease in quality in the untreated eggs and only a slight decrease with those oil treated. Most of the off-odors which developed in the eggs during storage, noticeable during the preparation, were not detectable in the baked cakes.

SUMMARY

It was found that the higher the candled quality and broken out quality of eggs, the more satisfactory was the resultant cake. Correlation coefficients were calculated and show close relationships between albumen index and angel food cake quality measurements. Volume and penetrometer measurements and taste panel scores for tenderness of angel food cake were closely correlated with change in interior egg quality as measured by albumen index. Cold storage temperature and controlled humidity storage maintained egg quality at a sufficiently high level to produce satisfactory angel food cakes for periods up to 6 months under the conditions of this study—the maximum length of storage used.

LITERATURE CITED

1. Barmore, Mark, A. Baking angel food cakes at any altitude. *Tech. Bull. No. 15*, Colorado State College (1935).
2. Barmore, Mark A. The influence of various factors, including altitude, in the production of angel food cake. *Tech. Bull. No. 15*, Colorado State College (1936).
3. Berg, L. R., and Bearse, G. E. The changes in egg quality resulting from force molting White Leghorn yearling hens. *Poultry Sci.*, **26**, 414 (1947).
4. Binnington, D. S., and Geddes, W. F. An improved wide-range volume measuring apparatus for small loaves. *Cereal Chem.*, **15**, 235 (1938).
5. Burke, Edith, and Niles, Kathryn B. A study of seasonal variation in egg white performance. *U. S. Egg and Poultry Mag.*, **42**, 542 (1936).

6. Clinger, Caroline, Young, Arlene, Prudent, Inez, and Winter, A. R. The influence of pasteurization, freezing, and storage on the functional properties of egg white. *Food Technol.*, **5**, 166 (1951).

7. Dawson, Elsie H., and Harris, Betsy L. Sensory methods for measuring differences in food quality. Review of literature and proceedings of conference. *Agric. Inf. Bull. No. 34*, U. S. Department of Agriculture (1951).

8. Grewe, Emily, and Child, Alice, M. The effect of acid potassium tartrate as an ingredient in angel food cakes. *Cereal Chem.*, **7**, 245 (1930).

9. Harns, J. Vivian, Sauter, E. A., McLaren, Barbara A., and Stadelman, W. J. The use of angel food cake to test egg white quality. *Poultry Sci.*, **31**, 1083 (1952).

10. Henry, W. C., and Barbour, A. D. Beating properties of egg white. *Ind. and Eng. Chem.*, **25**, 1054 (1933).

11. Kahlenberg, O. J. Preliminary studies on factors affecting egg white quality. *Bakers Digest*, **22**, 117 (1948).

12. King, Florence B., Norris, H. P., and Whiteman, Elizabeth, F. Some methods and apparatus used for measuring the quality of eggs for cake making. *Cereal Chem.*, **13**, 37 (1936).

13. Lee, Everett H. Frozen egg quality. *Bakers Digest*, **20**, 138 (1946).

14. MacDonnell, L. R., Hanson, H. L., Silva, R. B., Lineweaver, Hans, and Feeney, R. E. Shear—not pressure—harms egg white. *Food Inds.*, **22**, 273 (1950).

15. MacDonnell, L. R., Lineweaver, H., and Feeney, R. E. Chemistry of shell egg deterioration: Effect of reducing agents. *Poultry Sci.*, **30**, 856 (1951).

16. Miller, Elsie Lee, and Vail, Gladys E. Angel food cakes made from fresh and frozen egg whites. *Cereal Chem.*, **20**, 528 (1943).

17. Platt, W., and Kratz, A. Measuring and recording some characteristics of test sponge cakes. *Cereal Chem.*, **10**, 73 (1933).

18. Slosberg, Harry M., Hanson, Helen L., Stewart, George F., and Lowe, Belle. Factors influencing the effects of heat treatment on the leavening power of egg white. *Poultry Sci.*, **27**, 294 (1948).

19. Thelan, Roman J. Basic points in making better angel food cakes. *Bakers Digest*, **20**, 73 (1946).

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF BEEF DEHYDRATION

I. RATE OF DEHYDRATION AND STRUCTURAL CHANGES IN RAW AND COOKED MEAT*

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Small amounts of dehydrated meat were produced experimentally in the United States during the first World War and subsequently for several Arctic expeditions. Because of the need to conserve shipping space during the second World War attention was again directed to the dehydration of meat in 1942. Several commercial methods were developed and large quantities of dehydrated pork were produced in this country for Lend-Lease purposes, and in the United Kingdom and South America for use in England *(1, 5, 6, 7)*.

Extensive studies were carried out on methods of packaging *(10)* and on the vitamin content of the commercially produced dehydrated meat *(9)*. Although it filled a real need during the emergency, the quality of this product left much to be desired. It was felt that a histological and histochemical study of the changes which occur during the dehydration and rehydration of slices of meat would be of help in devising methods of producing dehydrated ground meat of better quality. Also, such a study might furnish basic information that would make it possible to produce satisfactory dehydrated steaks, chops, etc.

This paper presents the results of preliminary experiments to determine some of the structural and chemical changes occurring when slices of raw and pre-cooked muscle tissue were dehydrated at elevated temperatures. Other papers in the series will present the influence of such factors as carcass grade, type of muscle, and pretreatments of various types on the changes taking place during dehydration of beef.

MATERIALS AND METHODS

In the first of the 4 experiments in this series, raw and pre-cooked strips of *Biceps femoris* from a U. S. commercial grade cow, approximately 2 days after slaughter, were used; in the second experiment, samples of the same muscle were taken from a U. S. utility grade carcass. For experiments 3 and 4, strips of meat were removed from the *Biceps femoris* of light-weight U. S. commercial grade carcasses. All raw samples in these experiments were approximately 2½ inches x 1 inch x ¼ inch in size.

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Samples with muscle fibers oriented longitudinally and transversely were provided for both the raw and pre-cooked meat.

Samples (2-inch cubes) were pre-cooked in a steam (flowing) bath for about 45 minutes. The meat was then sliced into thin strips ½ inch thick.

Dehydration was done in a forced draft air oven at 70°C. or in a vacuum oven at 65°C. with more than 25 inches Hg vacuum at 65°C. for periods ranging from ½ to 24 hours. Samples were removed from the dehydrating chamber at predetermined periods for weight determinations, histological processing, or rehydration.

Rehydration of the dehydrated meat was done by placing the dehydrated samples in an excess of water in a covered dish at 70°C. for 15 minutes. The strips were blotted with filter paper before they were weighed to determine the degree of water absorption.

Per cent moisture of the raw meat was calculated on a 5-g. ground sample completely dehydrated at 65°C. in a vacuum oven. This served as a basis for all subsequent calculations in regard to moisture content of the dehydrated and rehydrated samples.

Due to the physical nature (hardening) of dehydrated muscle tissue, a technique for cutting dehydrated meat in paraffin blocks was devised. The procedure was to coat the surface of the tissue block with a thin layer of melted paraffin by a quick swab with a paraffin-soaked brush. After a few seconds, the block was ready to be cut. The procedure was repeated for each section. The sections so obtained were attached to slides individually (2 or 3 to a slide) and flattened out on an electric slide warmer. Two or three such slides were usually made for each sample to be used for histological and histochemical determinations.

The standard celloidin embedding technique was used also for most of the material processed by the modified paraffin embedding technique just described. No fixative except alcohol was used on any of the dehydrated samples. They were placed in absolute alcohol as the first step in both of the embedding techniques, but raw, pre-cooked, and rehydrated samples were fixed in Zenker-formol, prior to histological treatment through graded alcohol and ether. Preliminary investigations showed that these techniques induced little or no change in the histological characteristics of the tissue.

Celloidin sections of all samples were cut at 10 μ and stained with hematoxylin and Van Giesen's. All treated samples intended for celloidin embedding were given 1 to 2 days longer than required for normal tissues in each reagent following absolute alcohol to insure proper infiltration. All samples to be paraffin-embedded passed from absolute alcohol through the following reagents: 50% absolute alcohol and 50% Xylol (2 changes), 5 hours; 1 part soft and 2 parts hard paraffin (2 changes), 3 hours; embedded in 1 part soft and 2 parts hard paraffin (approximate melting point of the mixture, 62°C.).

Muscle fiber diameter in longitudinal sections (all fibers from one side to the other of a complete section) was measured directly with the aid of an ocular micrometer at 430× magnification. In transverse sections fiber diameter (50-100 fibers at random) was measured on the ground glass plate of a photomicrographic camera with the aid of a planimeter. The measurements were checked further on those samples which were photomicrographed later.

Sections of raw, cooked, and dehydrated meat were tested for the localization of potassium according to the method of Carere-Comes *(4)*. Similar sections were also tested for sulfhydryl groups by the method of Bourne *(3)* and for free α-amino groups by the ninhydrin reaction *(2)*.

RESULTS

The influence of two methods of dehydration and the type of slice (parallel or perpendicular to muscle fibers) on the rate and extent of moisture removal from slices of beef muscle tissue may be seen from the data (Table 1). In all samples the rate of moisture removal was slow. Although the data are too limited to permit a positive statement, the results indicate that less moisture was removed from the tissue of the commercial grade cow than from the tissue of younger animals under the same conditions of dehydration. No appreciable difference was found in meat dehydrated at 65°C. under vacuum and that dried at 70°C. in a mechanical convection air oven. Slices of tissue cut perpendicular to the muscle fibers dehydrated to a greater extent than did slices cut parallel to the muscle fibers. Considerable moisture was lost from the tissue during cooking and slices from pre-cooked meat dehydrated more rapidly

than comparable samples of raw tissue. None of the dehydrated slices of meat rehydrated to any appreciable extent under the conditions used for rehydration. Even treatment at higher temperatures and longer time failed to bring about increased water absorption of the dehydrated tissue. Since the ability to rehydrate is one of the requirements for a satisfactory dehydrated meat, it must be concluded that the dehydrated meat produced in these experiments was not an acceptable product from a practical standpoint. Consequently a study of the structural and chemical changes that occurred during the dehydration process should indicate changes that must be avoided if a satisfactory product is to be obtained.

TABLE 1

The moisture content of Biceps femoris muscle from different carcasses before dehydration,[b] after dehydration, and after rehydration

Experiment No.	Carcass and sample condition	Method of dehydration	Dehydration time (hr.)	Moisture content %			
				After dehydration		After rehydration	
				Long.	Trans.	Long.	Trans.
I	Commercial grade cow, raw	Air—70°	20.0	25.3	.....	.....	.....
		Vacuum—65°	20.0	29.2	.....	.....	.....
II	Utility grade, raw	Air—70°	5.0	15.7	13.8	.....	.....
			20.0	2.1	0.0	.....	.....
		Vacuum—65°	5.0	22.6	0.0	.....	.....
III	Lightweight commercial grade, No. 1, cooked	Air—70°	0.5	49.0	45.6	52.7	52.0
			1.5	33.9	28.0	38.6	37.0
			3.0	31.3	23.0	36.2	31.0
			24.0	−13.5[c]	−8.0[c]	11.7	15.0
IV	Lightweight commercial grade, No. 2, raw	Air—70°	0.5	65.0	62.7	66.7	64.3
			1.5	59.1	54.6	61.2	54.5
			3.0	52.8	45.9	55.0	45.5
			24.0	5.8	1.7	18.5	15.5

[b] Per cent moisture content of the raw or cooked meat was 73.1 for Experiment I; 72.6 for Experiment II; 63.8 for Experiment III; and 73.3 for Experiment IV.

[c] These impossible values probably are due to less complete cooking and higher moisture content of cube from which sample was taken as compared to meat used for moisture determination.

Microscopically, dehydration of slices of muscle tissue resulted in shrinkage of muscle fibers and diminution of the endomysial spaces which normally separate and surround the muscle fibers (Table 2 and Figures 2, A and B). In both raw and cooked samples muscle fiber diameter measurements on transverse sections were consistently greater than those obtained from corresponding longitudinal sections. This difference is probably due to the fact that in a longitudinal section some fibers are cut near the periphery of the fiber. If muscle fibers were true cylinders, muscle fiber diameter measurements on longitudinal sections cut at random should theoretically be 79% of the value measured from transverse sections.[d] In agreement with moisture data, during

[d] The average chord length of an infinite number of parallel lines intersecting a circle may be calculated as follows:

For ¼ of a circle (1 quadrant)

$$\int_0^r y\,dx = A = \frac{\pi r^2}{4}$$

$$\text{Then } y_{av.} = \frac{\pi r^2}{4r} = \frac{\pi r}{4}$$

and the average chord length is $\frac{2\pi r}{4} = \frac{\pi r}{2}$ Since the diameter is 2r, then the average chord length in terms of the diameter is $\frac{\pi r}{2}/2r \times 100 = 79\%$.

dehydration there was usually more fiber shrinkage in transverse slices than in corresponding longitudinal slices. There were no consistent differences in muscle fiber diameter in the dehydrated meat due to method of dehydration (air or vacuum) or to the method used for embedding (celloidin or paraffin). Muscle fiber diameter shrinkage during the early stages of dehydration was more rapid for pre-cooked samples than for raw samples. Although there was little increase in muscle fiber diameter when the dehydrated samples were placed in water there was some tendency for muscle fibers in the transverse slices to enlarge slightly.

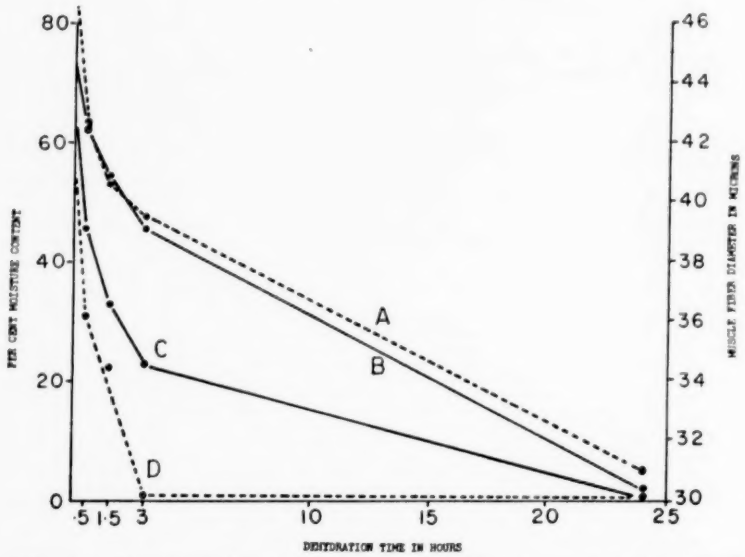

Figure 1. The progressive change in moisture content (————) and muscle fiber diameter (--------) in raw (curves A and B) and pre-cooked (curves C and D) transverse slices of Biceps femoris dehydrated at 70°C.

As mentioned above, dehydration of muscle slices, either raw or pre-cooked, resulted in diminution of the endomysial spaces as well as in fiber shrinkage. Furthermore, this decrease in endomysial space usually was complete in completely dried tissue, and the individual fibers often lost their identity and became merged. Also, dehydration effected a gradual loss of both longitudinal and transverse striations (Figure 2, E). Longitudinal striations disappeared quite early in the dehydration period. Transverse striations remained discernible longer, and in completely dehydrated samples a few muscle fibers still retained very faint transverse striations.

The nuclei of dehydrated muscle tissue stained poorly, if at all, particularly in sections from celloidin embedded blocks. The collagenous fibers and fat cells of the connective tissue of dehydrated tissue stained quite normally and did not appear to be much affected by loss of water.

Histochemically the only significant dehydration effect that could be established was the relocation of potassium. In normal raw or cooked tissue, potassium was rather uniformly distributed throughout the muscle fibers with some apparent concentration along the dark anisotropic bands (Figure 2, C). After dehydration potassium usually was found concentrated near the periphery of the muscle fibers (Figure 2, D). Apparently potassium moved with the water to the periphery of the fibers but was not able to move through the membrane at the muscle fiber-connective tissue boundary. Occasionally there were indications that potassium migrated into the connective tissue

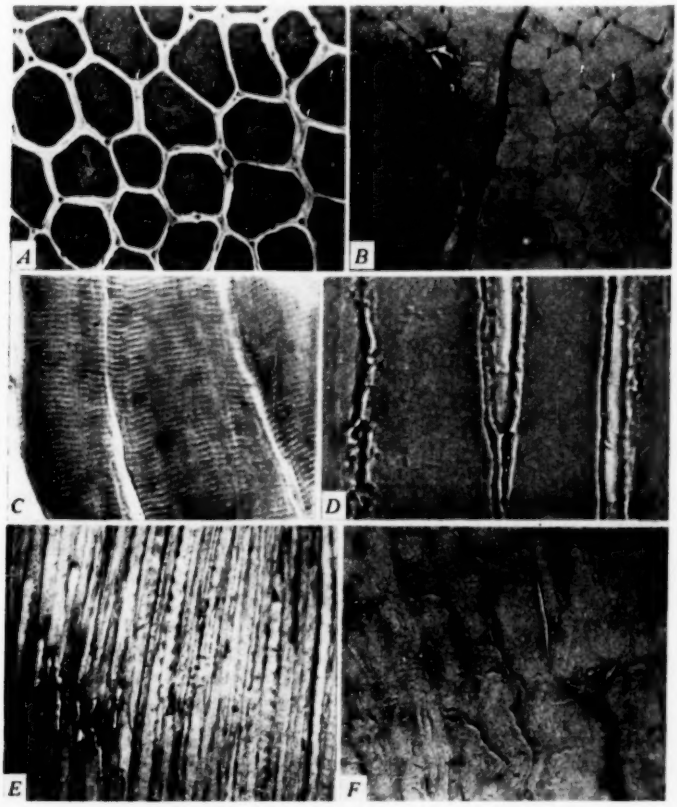

Figure 2. A. Transverse section of normal Biceps femoris, Zenker-formol fixed. × 170.

B. Transverse section of Biceps femoris, dehydrated from pre-cooked for 20 hours at 70°C. × 170. Compare A and B for size (diameter) of muscle fibers, amount of endomysial space, and the appearance of longitudinal striations, which were present in normal muscle tissue but completely absent after dehydration.

C. Frozen section of normal Biceps femoris stained with sienna orange for potassium. × 480. Transverse striations are clearly marked by dark orange bands with no indication of peripheral potassium localization.

D. Pre-cooked Biceps femoris dehydrated for 5 hours at 70°C. × 480. A case of extreme diminution of striations with very pronounced peripheral concentration of potassium as indicated by dark (originally orange) lines along the fiber boundaries.

E. Longitudinal section of a completely dehydrated Biceps femoris. × 40. Note extreme fiber shrinkage and elimination of endomysial space with consequent muscle fiber packing and merging, disappearance of both longitudinal and transverse striations, presence of partially collapsed autolytic gaps (lower left), and the failure of the nuclei to stain. (The lack of uniformly sharp focus was not due to technical error, but rather owing to the unique nature of the denatured muscle fibers.)

F. Longitudinal section of a rehydrated Biceps femoris sample after being dried for 24 hours at 70°C. × 170. Note the extensive fiber merging and the absence of transverse striations in most of the fibers.

where autolytic breaks were present in the muscle fibers. Pre-cooking, or time, temperature, and method of dehydration had no observable influence on the potassium distribution pattern in the dehydrated tissue. Since very few of the samples exhibited any appreciable water uptake when held in water, no definite conclusions could be reached concerning the possible redistribution of potassium during rehydration.

Sulfhydryl group and α-amino-acid determinations by the histochemical methods used were unsatisfactory and inconclusive. Thus it is impossible to state whether or not dehydration influenced these constituents of beef muscle tissue.

DISCUSSION

The fact that muscle fibers shrink during dehydration suggests that there may be a direct relationship between fiber (diameter) shrinkage and moisture loss during dehydration at the temperatures used in this study. Actually moisture loss and reduction in fiber diameter were quite parallel during the dehydration of raw meat, but muscle fiber shrinkage was comparatively more rapid than moisture loss during the early stages of dehydration of pre-cooked *Biceps femoris* (Figure 1). This difference in behavior of raw and cooked samples perhaps may be related to two changes that occurred during cooking: (a) some of the moisture was lost from the meat during cooking; and (b) the protein of the fibers undoubtedly heat-denatured to some extent, thus modifying its water-binding capacity. In any case, the results reported here indicate that the shrinkage of fibers occurring during dehydration is related directly to the moisture loss from the tissue. Actually a correlation coefficient of .65 between moisture loss and fiber diameter decrease was obtained for the series of 32 raw and pre-cooked samples reported here. This suggests that the moisture loss accounts for about half of the total factors influencing fiber shrinkage during dehydration by the methods used in this study.

In agreement with previous work on the dehydration of minced meat *(6)*, the rate of drying was rapid during the early stages of dehydration and became progressively slower as dehydration progressed.

The fact that, for both raw and pre-cooked samples, transverse slices dried more rapidly than longitudinal slices during the early stages of dehydration readily is accounted for if it can be assumed that water movement parallel to the fiber direction may be more rapid than diffusion perpendicular to the muscle fibers. Thus, during the early stages of dehydration of transverse slices the rate of drying would be limited by the speed of water movement to the surface through the endomysial spaces but later the slow diffusion through the fiber membrane would become limiting.

The disappearance of longitudinal and cross striations during dehydration would be expected if the physico-chemical structure of muscle fibers proposed by Szent-Györgyi *(8)* is correct. Szent-Györgyi believes that longitudinal striations as observed by histologists are artifacts resulting from the transverse disintegration of the three-dimensional gel structure within the fiber. This frequently is induced by the fixation techniques commonly employed in the histological processing of tissues. Since even partial dehydration undoubtedly would modify the gel structure within the fiber it is not surprising that the longitudinal striations largely disappeared during the early stages of dehydration. On the other hand, the

TABLE 2

The muscle fiber diameter of Biceps femoris muscle from different carcasses before dehydration, after dehydration, and after rehydration

Carcass	Embedding technique	Sample condition	Method of dehydration	Dehydration time	Type of section	Fiber diameter (μ)		
						Before dehydration	After dehydration	After rehydration
				hrs.				
Commercial grade cow	Paraffin	Raw	Air—70°	5.0	Long.	50.8	39.3	39.0
				5.0	Trans.	54.0	37.0	46.0
		Cooked	Air—70°	5.0	Long.	54.4	.....	40.0
				5.0	Trans.	50.0	41.0	40.0
			Vacuum—65°	5.0	Trans.	50.0	40.0	40.0
				20.0	Trans.	50.0	40.0	.....
Utility grade	Celloidin	Raw	Air—70°	5.0	Long.	44.3	32.8	32.8
				5.0	Trans.	50.0	34.0	37.0
				20.0	Long.	44.3	39.0	30.4
				20.0	Trans.	50.0	38.0	31.0
			Vacuum—65°	5.0	Long.	44.3	32.7	34.3
				5.0	Trans.	50.0	35.0	38.0
		Cooked	Air—70°	5.0	Long.	42.1	40.5	38.0
				5.0	Trans.	46.0	42.0	38.0
				20.0	Long.	42.1	35.0	38.6
				20.0	Trans.	46.0	42.0	37.0
			Vacuum—65°	5.0	Long.	42.1	32.5	40.4
				5.0	Trans.	46.0	39.0	39.0
	Paraffin	Cooked	Air—70°	20.0	Long.	42.7	37.5	31.3
				20.0	Trans.	46.0	30.0	36.0
			Vacuum—65°	5.0	Long.	42.7	37.8	34.7
				5.0	Trans.	46.0	42.0	37.0
				0.5	Long.	39.7	35.8	36.0
				0.5	Trans.	41.0	36.2	37.3
Lightweight commercial grade, No. 1	Celloidin	Cooked	Air—70°	1.5	Long.	39.7	32.0	31.7
				1.5	Trans.	41.0	35.4	32.8
				3.0	Long.	39.7	29.8	30.5
				3.0	Trans.	41.0	29.9	33.4
				24.0	Long.	39.7	29.8	30.5
				24.0	Trans.	41.0	31.0	30.9
Lightweight commercial grade, No. 2	Celloidin	Raw	Air—70°	0.5	Long.	44.7	37.2	36.8
				0.5	Trans.	46.6	42.7	44.2
				1.5	Long.	44.7	38.4	35.6
				1.5	Trans.	46.6	40.6	43.7
				3.0	Long.	44.7	38.8	35.5
				3.0	Trans.	46.6	39.5	42.8
				24.0	Long.	44.7	29.2	30.4
				24.0	Trans.	46.6	31.0	37.0

cross striations, according to Szent-Györgyi, represent a periodic variation of physico-chemical structure along the actomysin cylinder, and therefore would be expected to be more stable to moisture loss. This was found to be true since the cross striations remained more or less definite through most of the dehydration period and still could be distinguished in some fibers of completely dehydrated tissue (Figure 2, E).

The "merging" of muscle fibers during dehydration is not so readily explained. Since the sarcolemma surrounding each muscle fiber is a thin but discrete membrane, it would not be likely to disappear completely during dehydration. Actually it is quite possible that the observed merging of fibers does not involve direct contact between the sarcolemma of adjacent fibers but only a change in the structural organization of the fibrous net surrounding the sarcolemma. In this way the boundary membranes surrounding each muscle fiber might seem to disappear only because they shrink to a size too small for optical microscopic resolution. Under the conditions of rehydration used here there apparently was not enough water absorption by the boundary membranes to bring about the reappearance of muscle fiber boundaries (Figure 2, F).

SUMMARY

Longitudinal and transverse strips of beef *Biceps femoris*, both raw and pre-cooked, from different carcasses were dehydrated at either 70°C. in a mechanical convection oven or at 65°C. in a vacuum oven for varying periods of time up to 24 hours.

Loss of water from the tissue was accompanied by diminishing endomysial (and perimysial) spaces, progressive reduction in muscle fiber diameter, disappearance of longitudinal striations, decreasing distinctness of cross striations in muscle fibers, and movement of potassium to the periphery of the muscle fibers.

For both raw and pre-cooked meat moisture loss was rapid during the early stages of dehydration and became progressively slower as dehydration progressed. Pre-cooked meat exhibited a more rapid loss of moisture during the early stages of dehydration and dehydrated to a lower moisture level than did raw meat.

Dehydrated meat held in water at 70°C. for 15 minutes did not absorb much water regardless of the type of strip dehydrated or the method or extent of dehydration. In some cases the partially rehydrated samples, as compared to the dehydrated meat, exhibited a slight increase in muscle fiber diameter, partial return of endomysial spaces and greater distinctness of cross striations.

LITERATURE CITED

1. Bate-Smith, E. C., Lea, C. H., and Sharp, J. G. Dried Meat. I. *J. Soc. Chem. Ind.*, **62**, 100-104 (1943).

2. Berg, W. Zum Histologischen Nachweis der Eiweisspeicherung in der Leber. *Arch. ges. Physiol. Pflügers*, **214**, 243-249 (1926).

3. Bourne, G. Mitochondria, Golgi apparatus and vitamins. *Australian J. Exptl. Biol. Med. Sci.*, **13**, 238-295 (1935).

4. CARERE-COMES, O. Neue Methoden zum Histochemischen Nachweis des Kaliums und zur Elektiven Färbung der Kalireichen Gewebe. *Z. wiss. Mikroskop.*, **55**, 1-7 (1938).

5. Committee on Dehydration of Meat. United States Department of Agriculture. Meat Dehydration. *Circular 706*, U. S. Department of Agriculture (1944).

6. EDE, A. J., AND PARTRIDGE, S. M. Dried Meat. IV. The effect of some physical factors on the rate of drying of minced meat in heated air. *J. Soc. Chem. Ind.*, **62**, 194-200 (1943).

7. KRAYBILL, H. R. Dehydration of meat. *Ind. and Eng. Chem.*, **35**, 46-49 (1943).

8. SZENT-GYÖRGYI, A. G. Chemistry of Muscular Contraction. 2nd ed., 1951, Academic Press, Inc., New York, N. Y.

9. WHITMORE, R. A., POLLARD, ANNE EARLE, KRAYBILL, H. R., AND ELVEHJEM, C. A. Vitamin content of dehydrated meats. *Food Research*, **11**, 419-424 (1946).

10. WHITMORE, R. A., SELIGSON, DAVID, KRAYBILL, H. R., AND WEBB, B. H. Packaging dehydrated meat. *Food Research*, **13**, 19-28 (1948).

STORAGE CHANGES IN THE PHOSPHORUS, NITROGEN, AND FATTY ACID CONSTITUENTS OF THE LIPID IN CANNED FLORIDA VALENCIA ORANGE JUICE

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The lipid component of orange juice has received considerable attention because of its possible influence on flavor changes which develop in the canned juice during storage. Several investigators *(2, 4, 5, 7, 8, 9, 10, 11)* have presented analytical data, both qualitative and quantitative, on this fraction. Huskins, Swift, and Veldhuis *(4)* analyzed the lipids from canned pasteurized orange juice stored for 2 years under normal climatic conditions at Winter Haven, Florida (annual mean temperature about 22°C.). They found that the phosphorus had decreased to one-tenth of its original value and the nitrogen to one-fifth, choline having entirely disappeared. Also, the amount of lipid which could be extracted from the stored juice had decreased. They concluded that the phosphatides had hydrolyzed and that the choline and ethanolamine, as well as part of the phosphorus, had been lost to the aqueous phase of the juice. These investigators also noted changes in the fatty acid constituents, which could have been the result of oxidation. In this previous work, no attempt was made to establish whether these changes occurred rapidly or slowly. The present analyses of the lipid from pasteurized, stored Valencia orange juice were carried out to study this question. Attention was given only to the changes in the phosphorus, nitrogen, and fatty acid constituents.

EXPERIMENTAL

Extraction of lipids. Fresh Valencia orange juice was obtained from a local cannery in 1950. In the laboratory it was mixed, pasteurized at 88°C., canned, and quickly cooled.

About one-third of the cans were held at 0-5°C. for 2 days until the lipid could be extracted. The extraction was carried out by passing the juice through an Oliver Precoat filter[b] and then extracting the lipid from the filter-aid with acetone as described by Huskins, Swift, and Veldhuis *(4, 5)*. The solvent was evaporated and the lipid taken up in petroleum ether. This step eliminated the water which the acetone also extracts from the damp filter-aid. The petroleum ether solution of the lipids was then stored under refrigeration (0-5°C.) until the analysis could be made.

The remaining two-thirds of the cans were stored in the laboratory loft. After storage periods of 6 and 12 months, portions of the stored juice were processed for lipids as outlined above.

Prior to analysis, the petroleum ether solutions of the lipids were treated as follows: each solution, in a 3-necked flask equipped with a mechanical stirrer, condenser, and vacuum gauge, was warmed over a steam bath to evaporate most of the solvent at atmospheric pressure. Then the flask was immersed in cold water, the stirrer was started,

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[b] The mention of trade products does not imply that they are preferred by the Department of Agriculture over similar products not mentioned.

and vacuum applied and maintained at 10 mm. Hg pressure while the water bath was slowly heated (45 minutes) to boiling, and for 15 minutes thereafter.

Analytical methods. The total lipid was determined by the method of Swift *(12)* on the juices initially and after 12-months' storage. Total nitrogen was determined by the Kjeldahl method and phosphorus by ashing the lipid with magnesium nitrate, precipitating as ammonium phosphomolybdate, and then titrating with standard alkali. Amino nitrogen was determined on an acid hydrolysate of the lipid by the Van Slyke method *(14, 15)* and the choline on an aliquot of the same solution by the method of Thornton and Broome *(13)*. See Table 1.

TABLE 1

Effect of orange juice storage on the nitrogen and phosphorus content of the juice lipid

Constituent	Initial	After Storage	
		6 months	12 months
	%	%	%
Total nitrogen	0.73	0.52	0.44
Choline nitrogen	0.27	0.11	0.08
Amino nitrogen	0.25	0.14	0.18
Other nitrogen (by difference)	0.21	0.26	0.18
Total phosphorus	1.40	0.77	0.63

The fatty acids were obtained from the lipid in the usual manner by saponification, extraction of the unsaponifiable substances with ethyl ether, acidification of the aqueous residue, and extraction of the fatty acids with petroleum ether. After evaporation of the solvent, the fatty acids were examined spectrophotometrically using the method of Brice, Swain, Schaeffer, and Ault *(1)*. Iodine numbers were determined by the Wijs (30 min.) method (Table 2).

TABLE 2

Effect of orange juice storage on the fatty acids of the juice lipid, calculated on the whole lipid basis

Constituent	Initial	After Storage	
		6 months	12 months
	%	%	%
Fatty acids [c]	57.55	59.51	64.24
Conjugated			
Dienes	0.87	0.84	1.19
Trienes	0.017	0.012	0.004
Tetraenes	0.005	0.000	0.02
Non-conjugated			
Arachidonic	0.000	0.000	0.000
Linolenic	3.98	4.36	4.70
Linoleic	17.35	17.50	17.52
Oleic and palmitoleic	23.50	22.60	24.48
Saturated	11.83	14.20	16.33

[c] Iodine value (Wijs, 30 min.) was initially 115.0. Corresponding iodine value for the fatty acids from juice stored 6 months was 110.1 and for those from juice stored 12 months, 106.7.

RESULTS AND DISCUSSION

Lipid loss on storage. The yields of lipid isolated were not measured. Instead, analytical determinations were made on the fresh juice and on the juice after 12-months' storage as noted above. The results showed that the lipid percentage had fallen from 0.069 to 0.059 in this period, the loss probably being from the phosphatide part of the lipid as noted below.

Changes in the phosphatides. Table 1 shows that the principal change, as far as the nitrogen and phosphorus are concerned, is a loss, particularly for choline nitrogen. No reason can be advanced at this time for the apparent slight increase in amino nitrogen during the second 6-months' storage period. Puzzling also is the more rapid loss of phosphorus as compared to total nitrogen since the phosphorus is generally considered as the link that connects the nitrogenous constituent to the rest of the phosphatide molecule. Moreover, the ester linkage between the phosphorus and glycerol is generally thought to be more resistant to hydrolysis than is the one between phosphorus and choline or other nitrogenous constituent. Davis and Gill *(3)* have reported a recombination between freed nitrogenous constituents and the remaining parts of phosphatide molecules after the loss of phosphorus by hydrolysis. However, such a recombination here seems improbable since the nitrogenous constituents, once set free from the phosphatide, would then be present in the aqueous part of the juice, and might well be bound in that phase by the great excess of relatively strong organic acids.

Changes in the fatty acids. A comparison of the data in Table 1 with those of Table 2 shows that the fatty acids of the lipid underwent much less drastic changes than did the phosphorus- and nitrogen-containing parts of the phosphatides. These latter changes, which represent considerable loss of lipid, account for most of the increase in fatty acids since these are expressed as percentages of the whole lipid. The decrease in iodine value shows an overall loss in unsaturation. After 12 months' storage, the diene-conjugated acids showed an increase.

SUMMARY

During the storage of orange juice at room temperature, it was found that there was a considerable loss of nitrogen from the lipid fraction over a period of one year. This loss was particularly severe with choline nitrogen, the other forms suffering to a lesser extent. Also noted was a loss of approximately half of the phosphorus, most of it during the first 6 months. There was an overall increase in the percentage of fatty acids in consequence of the loss of those portions of the phosphatides associated with the nitrogen and phosphorus. The only other noteworthy changes in the fatty acids were a decrease in iodine value and a slight increase in diene conjugation.

LITERATURE CITED

1. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C. Spectrophotometric determination of small proportions of polyunsaturated constituents in fatty materials. *Oil & Soap*, **22**, 219 (1945).
2. Curl, A. L., and Veldhuis, M. K. The origin of the off-flavor which develops in processed orange juice. *Fruit Products J.*, **26**, 329 (1947).
3. Davies, W. L., and Gill, E. Investigations on fishy flavour. *J. Soc. Chem. Ind. (London)*, **55**, 141 T (1936).
4. Huskins, C. W., Swift, L. J., and Veldhuis, M. K. Constitution of the lipid from stored Florida Valencia orange juice. *Food Research*, **17**, 109 (1952).
5. Huskins, C. W., and Swift, L. J. Changes in the lipid fraction of Valencia orange juice during pasteurization. *Food Research*, **18**, 305 (1953).
6. Jamieson, G. S. *Vegetable Fats & Oils.* 2nd Ed., 1943, Reinhold Publishing Corp., New York, N. Y.

7. MATLACK, M. B. The fatty constituents of California Valencia orange pulp (Citrus aurantium sinensis L.). *J. Org. Chem.*, **5**, 504 (1940).

8. NOLTE, A. J., AND VON LOESECKE, H. L. Chemical and physical characteristics of the petroleum ether soluble material of fresh and canned Florida Valencia orange juice. *Food Research*, **5**, 457 (1940).

9. SWIFT, L. J. Fatty acids of the lipids from freshly canned Florida Valencia orange juice. *Food Research*, **17**, 8 (1952).

10. SWIFT, L. J., AND VELDHUIS, M. K. Constitution of the juice lipid of Florida Valencia orange (Citrus sinensis L.). *Food Research*, **16**, 142 (1951).

11. SWIFT, L. J. Flavor changes in stored canned orange juice. *Proc. Florida State Hort. Soc.*, **64**, 181 (1951).

12. SWIFT, L. J. The determination of crude lipid in citrus juices. *J. Assoc. Offic. Agr. Chemists*, **29**, 389 (1946).

13. THORNTON, M. H., AND BROOME, F. K. Determination of choline, a photometric modification of Beattie's method. *Ind. Eng. Chem., Anal. Ed.*, **14**, 39 (1942).

14. VAN SLYKE, D. D. The gasometric determination of aliphatic amino nitrogen in minute quantities. *J. Biol. Chem.*, **16**, 121 (1913).

15. VAN SLYKE, D. D. Note on the micro-method for gasometric determination of aliphatic amino nitrogen. *J. Biol. Chem.*, **23**, 407 (1915).

FIBER DIAMETER IN RELATION TO TENDERNESS OF BEEF MUSCLE[a, b]

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"Tenderness" is defined by Webster as "easily impressed, broken, cut, masticated . . . ." Easily impressed, broken, cut, masticated is the hope of all beef consumers. Market beef is classed by sex and age, graded for quality, fatness and conformation, and cut into retail portions, largely on the basis of its expected tenderness. Cook books describe dry heat, broiling, frying and roasting, as suitable for meat with natural tenderness. They give detailed directions for using moist heat to tenderize meat cuts that lack natural tenderness. Consumers, of course, also desire high food value, juiciness and good flavor in beef, but their first consideration is for beef that can be "easily impressed, broken, cut or masticated."

Market and consumer experience, through the years, has discovered several factors that may influence the tenderness of beef. (a) Beef from freshly slaughtered carcasses (one to three days after slaughter) is less tender than beef from the same carcasses that have been aged or hung to "ripen" in a 0.7 to 1.7°C. (33 to 35°F.) chill room for 5 days or more. Normally beef aged for 10 days or longer is more tender than beef aged a shorter period. (b) Beef from young animals is more tender than beef from older ones. That means that beef from yearling and 2-year-old steers and heifers is usually more tender than that from older cows or bulls or even from 3-, 4-, and 5-year-old steers. (c) Beef from well-fed, well-fattened animals is usually more tender, and otherwise more desirable than thin beef from poorly fed animals. (d) Certain working muscles, such as those of the shank, neck, shoulder and round are less tender than the larger, less worked muscles of the back and loin.

Research has corroborated these opinions of the market and has determined many of the reasons for the differences noted. It is known that there are differences in the amount of connective tissue in animals of various ages and among the "working" and "less worked" muscles of the same carcass. It is recognized that muscle bundles swell or enlarge to some extent when animals fatten. Also aging, enzymes, acids and doubtless other agents change the character of muscle tissue. However, there is little information on the histology of muscle tissue as it relates to tenderness.

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Research has also discovered that there are differences in the tenderness of meat from animals that by breeding, feeding, age and method of handling should have been similar. If more tender beef is to result from research, it is essential to determine the inherent causes of tenderness or toughness in the fibers and connective tissue of beef, to learn to identify those differences and to be able to measure changes produced in them by experimental means in order to improve our beef supply. The purpose of this study is to record and compare the differences in fiber diameter from a wide variety of beef samples of known history and relate them to the previously determined tenderness of the heated meat *(8)*.

REVIEW OF LITERATURE

Texture in meat, firmness, and moistness are believed by many to be closely associated with tenderness. This means that in 2 similar cuts the finer textured meat would be more tender. Hirzel *(9)* stated that texture depends on the number and development of the fibers in a muscle bundle; whereas, tenderness depends upon the amount and distribution of connective tissue. Hammond *(6)* cited many authorities in his report on studies of the texture of meat. He found the consensus to be that the size of the muscle fiber increased with age, exercise, and nutrition; male animals have the largest fibers, castrated males intermediate, and females the smallest. As a result of this study of sheep muscle, he reported that post-natal growth influenced fiber and muscle bundle size. Those animals with the greatest post-natal growth had the largest fiber diameter and muscle bundles. He also reported that muscle bundles in the wether are larger than those in the ewe and that in the smaller sheep breeds there was finer grain to the lean meat; however, it was not in proportion to the differences in body weight. Robertson and Baker *(13)* in a study of level of feeding found that fiber diameter was largest in full-fed animals, intermediate for half-fed and smallest for those on roughage only. This would indicate a relationship between fiber size and muscular nutrition when animals of the same age are used. The size of muscle fibers is reported by Lehmann *(10)* as a factor contributing to tenderness in meat, the smaller the fiber the more tender the meat.

Brady *(2)* found significant differences in diameter of muscle fibers between grade yearling steers, of Hereford and Shorthorn breeding, and mature grade Holstein cows, and between fresh, aged and cooked muscles; but no difference in diameter of fibers from different muscles. This study was made on triceps brachii, longissimus dorsi, adductor and semitendinosus muscles. The difference in number of fibers making up a muscle bundle was reported by Brady to be significant between steers and cows and between different muscles, but he found a low correlation (+.22) between shearing strength and diameter of muscle fiber in aged muscle. There was a closer relationship in the cooked samples, the correlation coefficient being +.53. The number of fibers per bundle was more closely related to tenderness. His conclusions were that texture was dependent on size of bundle, the larger the bundle the finer the texture, and the finer the texture the more tender the meat. Beard *(1)* also concluded that the inherent properties of the endomusium contributed more to tenderness than size of fiber. On the other hand, Moran and Smith *(11)*, in studying characteristics of muscle fibers and tenderness, showed that the diameter of the muscle fiber, the area of cross-section of primary bundles and of secondary bundles was progressively larger in psoas major (tenderloin), longissimus dorsi (rib), biceps femoris (top round) and semimembranosus (inside round). Likewise, the number of fibers decreased for each primary bundle in the same order. This would indicate that small muscle fibers and small primary and secondary bundles are associated with tenderness, as the muscles are listed in decreasing order of tenderness. Ramsbottom, Strandine and Koonz *(12)* found great variations in texture of muscles as determined by the size of the bundles (fasciculi) and the amount of connective tissue (perimysium) surrounding the bundles. They found well defined fasciculi and extensive perimysium in the superficial pectoral muscles, whereas in the psoas major the connective tissue was in such small amounts that the muscle was not divided distinctly into bundles, the result being a muscle that was smooth and fine

textured. The former muscle had a high shearing strength value and was tough; the latter had a low value. This study was made on 25 of the larger muscles from each of three "U. S. Good" grade beef carcasses.

Meat from older, mature animals is generally considered to be less tender than that of younger, immature animals. Hiner and Hankins *(8)* found that the age of the animal at the time of slaughter had a significant effect on tenderness. Their study was based on 52 animals that varied from 10-week-old calves to 9-year-old cows. They found that veal calves were the most tender, and that tenderness decreased with increasing age of the animal. They also reported that tenderness in veal calves did not vary among the cuts as much as it did in the more mature animals. Beginning with 900-pound, 14-month-old steers, there were 4 distinct degrees of tenderness: (a) the most tender, the tenderloin; (b) club steak from the shortloin, sirloin steak from the loin end, prime rib steak and two chuck samples; (c) the round steak; and (d) the least tender, the neck and foreshank. Helser, Nelson and Lowe *(7)* reported that meat from veal calves was less tender than that of older animals; whereas, Foster *(5)* found no difference between meat from a year-old Choice steer and that from a steer 9 years old. However, the majority of the reports on the tenderness of meat indicated the general tendency was for it to vary inversely with age.

NATURE OF MUSCLE TISSUE

Muscle tissue performs work by contracting or by shortening and thickening of its constituents. Due to the innumerable types of work necessary in vertebrates, each muscle is constituted in such a manner as to best perform its function. There are 2 types of muscle, smooth and striated. Smooth muscle is, in most cases, involuntary and is found mainly in the internal organs. On the other hand, except for cardiac muscle, striated muscle is subject to voluntary control and is attached to the skeleton. These striated skeletal muscles are referred to as "meat" in the slaughtered, dressed and chilled animal carcass. Basically, all striated muscles are made up of primary muscle fiber bundles held together by connective tissue. Several primary muscle bundles in turn are bound together by connective tissue to form secondary bundles. Likewise, several secondary bundles are bound together to form a tertiary bundle, etc., until a large muscle is formed. Each muscle bundle is made up of a very large number of parallel independent muscle fibers held together by interstitial connective tissue or endomysium. Endomysium is a thin layer of collageneous tissue which may or may not also contain elastic fibers.

Muscle fibers in cross section are round or oval, but vary in diameter and length. Except for those fibers attached to a tendon, which are blunt, they tend to taper to a point. Surrounding each muscle fiber is a continuous, thin, transparent, structureless membrane, the sarcolema. A freshly separated muscle fiber appears slightly yellow and striated in both longitudinal and transverse directions. These striations are due to the arrangement and the optical properties of the myofibrils which are numerous very thin fibrils located in the "cell sap" or sarcoplasm of each muscle fiber. These thin fibrils lie parallel to one another and run the entire length of the fiber, thus accounting for the longitudinal striation. The myofibrils are not homogenous but consist of alternate dark and light disc-like sections which coincide in adjacent myofibrils and thus give the fiber its transverse striation. Each muscle fiber contains numerous nuclei generally located just beneath the sarcolema. The sarcoplasm contains a substance which consists of myosin and myogen, nucleoproteins, liposomes, salts and a pigment which is closely related to hemoglobin, known as myoglobin. There are also numerous metabolic intermediaries such as lactic acid and creatin-phosphate.

EXPERIMENTAL

Origin of meat samples. Meat samples used in this study were obtained from 52 beef and veal animals that had been slaughtered and chilled under uniform conditions. The 52 animals consisted of 8 cows that averaged approximately 5½ years of age; 8 barren heifers, 3 years old; twenty-five 900-pound steers, 14 months old; eight 500-pound steer calves, 7 months old; and 3 veal calves, 2 months of age. According to the U. S. grading standard in use prior to 1951, the carcasses graded as follows: cows, Commercial; heifers, Commercial; steers, Good; 500-pound calves, high Commercial; and veal calves,

Good. These cattle were all beef-type Shorthorns, milking type Shorthorns or crosses between the 2 types.

Preparation of samples for testing. After each carcass was aged for 12 to 15 days at 0.6 to 1.7°C. (33 to 35°F.) it was cut into its primal cuts. From these primal cuts nine 1½-inch pairs of samples were removed for study. These samples were distributed in such a manner that each primal cut was represented by one or more samples. The samples were removed at the following locations (Figure 1): (a) neck, 6th cervical

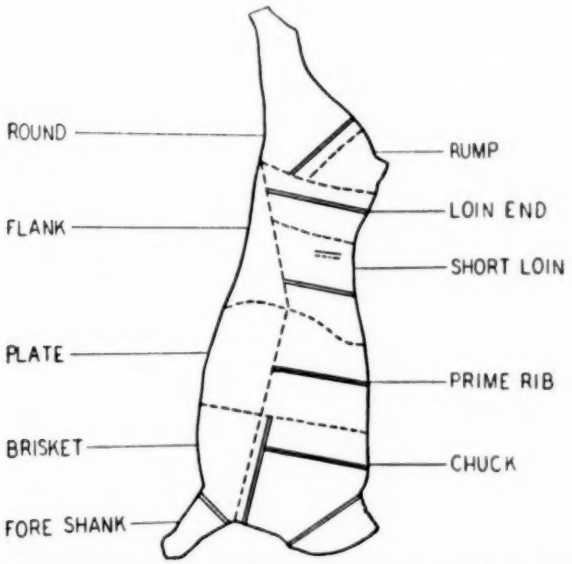

Figure 1. Side of beef, with primal cuts outlined by broken lines and locations of samples used in study by solid lines.

vertebra; (b) foreshank, through radius and ulna bone near elbow joint; (c) 3rd rib, at 3rd thoracic vertebra; (d) across humerus or round bone of chuck; (e) 8th rib, at 8th thoracic vertebra; (f) 3rd lumbar vertebra; (g) psoas major (tenderloin) below 6th lumbar vertebra; (h) rise of pelvic arch (loin end); and (i) 1½ inches from cut surface or face of the round. Each set of 9 samples was from one side, and each age group of animals was represented by an equal number of sets of samples from the right and left sides of the carcass. As soon as the samples were cut, they were returned to the refrigerator where they remained overnight.

As near the 14th day after slaughter as possible, the 9 prepared samples representing the right or left side were tested for tenderness, after heating to an internal temperature of 60°C. (140°F.). Prior to weighing and heating, small cubes of muscle approximately 12 mm., on a side were dissected and fixed for histological study.

The small cubes of muscle for histological studies were removed and treated in a uniform manner. This was done by selecting the muscle that made up the largest proportion of that sample. After the muscle was chosen, the area was selected from which the histological sample was to be removed. In order to avoid variables that may occur near the surface and in the center of the muscle, a location approximately one-third of the distance from the outer surface was used. This was determined by measuring the diameter of the muscle on its longest axis. As soon as the 12 mm. cube of meat was removed, it was placed in approximately 50 ml. of 10% formalin solution where it remained for 5 to 8 days.

The cube samples used for the histological study were removed from the following muscles, all of which are classified as voluntary and striated; neck, serratus ventralis; foreshank, deep digital flexor; 3rd rib of chuck, longissimus dorsi; cross-arm of chuck, triceps brachii; 8th rib, longissimus dorsi; shortloin, longissimus dorsi; tenderloin, psoas major; sirloin, gluteus medius; and round, semimembranosus, semitendinosus and biceps femoris muscles.

After fixing, the muscle samples were removed from the formalin solution and cut in halves lengthwise. One-half of each sample was trimmed into a rectangular block approximately 10 x 5 x 6 mm. The blocks of tissue were embedded in paraffin by the usual procedure of dehydrating in increasing concentrations of alcohol (70% to absolute), followed by xylene, xylene and paraffin; and finally embedded in paraffin. The second half of each sample was returned to the formalin solution for studies on fiber diameter.

For the fiber diameter study, a heavy circular line was drawn with a glass marking pencil, about 20 mm. in diameter, on a clean glass slide. Several drops of clear beef blood serum were placed within the circled area. The fixed sample of muscle was removed from the formalin solution, the excess moisture blotted off, and then, using a sharp razor blade, very thin sections of muscle tissue were sliced off and floated in the serum. With the use of a dissecting microscope and a pair of dissecting needles, a group of 30 to 40 fibers were teased from the slice of muscle. After removing any unused portion of the slice, a clean cover slip was carefully placed over the circular area containing the serum and individual fibers. The prepared slide was then transferred to a microscope equipped with an ocular micrometer.

The average diameter of fiber was determined by measuring 12 muscle fibers at random. Any fibers that appeared to be split or damaged in any way during the teasing operations were avoided. Each of the 12 fibers was measured at 3 locations, avoiding the ends which may be pointed or rather flat in character. The 36 measurements obtained in this manner were averaged and considered to be the average diameter of muscle fiber for that muscle. The measurements were recorded in microns.

RESULTS

The average diameter of the fiber for each of the 9 muscles from each age group is shown in Table 1 and Figures 2, 3, and 4. It will be noted that the fiber diameters for the 9 muscles in each age group classified themselves into 4 general groups. The neck and foreshank muscles had the largest diameter, closely followed by those of the round; third, the two chuck muscles, eighth rib, shortloin and loin end; and fourth and smallest, those of the tenderloin. This grouping was the same as that previously reported by Hiner and Hankins *(8)* for resistance to shearing for these same muscles. It is of interest to note that the diameters of fibers for chuck at third rib, eighth rib, and shortloin for each age group were quite similar. These 3 samples were from the longissimus dorsi muscle but were removed at locations representing approximately ¼, ½, and ¾ of its total length. The 3 muscles, semimembranosus, semitendinosus and biceps femoris varied little in average diameter of fiber for any age group.

It is also shown in Table 1 that as age of animal increased the diameter of fiber increased in all muscles studied. To determine if these differences were of any significance, the fiber diameter values were tested for significance of differences between means for each of the 5 age groups, using the "T" test, Fisher *(4)*. This analysis showed that the mean difference in fiber diameter was significant at the 1% level for each of the 9 samples between cows and 900-pound steers, cows and steer calves, heifers and steer calves and 900-pound steers and 500-pound steer calves. In general the differences in diameter of fiber between consecutive age groups were less

TABLE 1

Average diameter of muscle fibers from nine muscles of beef from animals of different ages and sexes [4]

Animals used	Number	Age of animal	Meat aging period	Diameter of fiber in—								
				Neck	Foreshank	Chuck		8th rib	Short loin	Tenderloin psoas	Loin end	Round
						3rd rib	Cross-arm					
		month	*days*	*microns*	*microns*	*microns*	*microns*	*microns*	*microns*	*microns*	*microns*	*microns*
Cows	8	67	13.0	69.6	71.2	61.1	60.4	61.6	61.9	47.2	61.7	64.7
Heifers, barren	8	37	13.8	65.3	68.3	58.8	58.8	58.2	58.8	45.3	58.6	62.8
Steers—900 pounds	25	14	13.4	58.9	62.1	54.5	53.9	56.0	55.6	41.7	55.6	57.1
Steer calves—500 pounds	8	8	12.8	51.2	52.1	42.4	41.6	43.4	44.2	31.1	43.7	46.5
Veal calves	3	2.5	13.7	50.5	49.9	37.3	40.4	39.1	38.6	26.1	39.8	46.0

[4] Muscles represented:
Neck ..... servatus ventralis
Foreshank ..... deep digital flexor
Third rib ..... longissimus dorsi
Cross-arm ..... triceps brachii
Eighth rib ..... longissimus dorsi
Shortloin ..... longissimus dorsi
Tenderloin ..... psoas major
Loin end ..... gluteus medius
Round ..... semimembranosus
semitendinosus
biceps femoris

TABLE 2

Percentage increase in muscle fiber diameter of the nine samples from veal calves to each succeeding age group

Animal group	Number	Age of animal	Meat aging period	Percentage increase from veal-calves to—								
				Neck	Foreshank	Chuck		8th rib	Short loin	Tenderloin	Loin end	Round
						3rd rib	Cross-arm					
		month	*days*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*
From veal calves to	3	2.5	13.7									
Steer calves—500 pounds	8	8	12.8	1.4	4.4	13.7	3.0	11.0	1.5	19.2	9.8	1.1
Steers—900 pounds	25	14	13.4	16.7	24.4	46.1	33.4	43.2	44.0	59.8	39.7	24.1
Heifers, barren	8	37	13.8	29.5	36.9	57.6	45.5	48.8	52.3	73.6	47.2	36.5
Cows	8	67	13.0	38.0	42.7	63.8	49.5	57.5	60.4	80.8	55.0	40.7

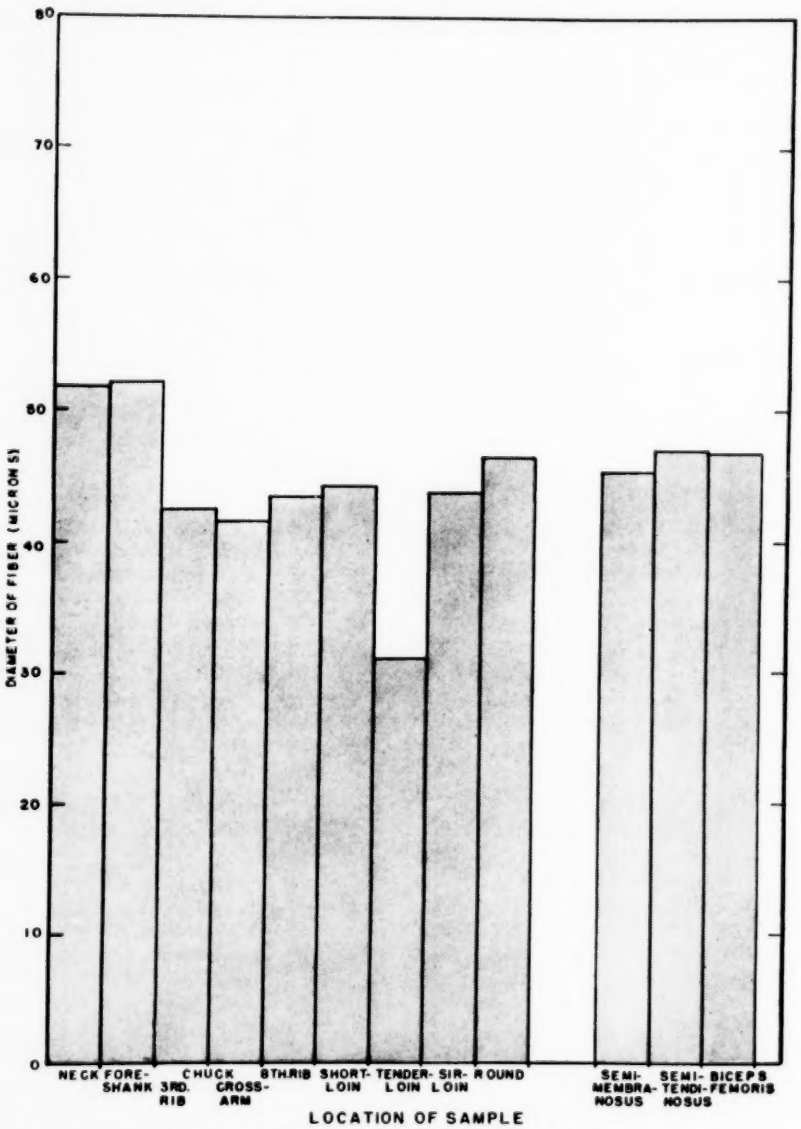

Figure 2. Relationship between fiber diameter of 9 muscle samples of beef and 3 muscles of the round from 500-pound steer calves.

significant than when there was an intervening age group, except between the 900-pound and 500-pound steer calves. In most cases the differences between consecutive age groups were significant at the 5% level, the remaining few not being significant. A similar analysis of the mean diameter of fibers for the 3 muscles of the round by age groups showed the same results.

This analysis of fiber diameters would indicate that the greatest change occurred between 8 and 14 months of age. The 500-pound steer calves represented 8-month-old animals and the 900-pound steers the age at which growth has begun to level off. The lack of more significant differences between veal calves and the other age groups of beef was probably due to small numbers *(3)* and the difficulty of teasing out the whole individual fibers for measurement.

As previously stated, data in Table 1 show that in all cases the diameter of fiber consistently increased with each age group. However, the semimembranosus muscle, one of the 3 round muscles studied, was slightly larger in veal calves than in 500-pound steer calves. The greatest difference in average diameter of fiber between veal calves and cows was 23.8 microns and the smallest was 18.4 microns. These differences were found in the longissimus dorsi muscle of the 3rd rib and in the semimembranosus muscle of the round, respectively. These differences between veal calves and each succeeding age group are shown in Table 2, expressed as percentage increase. It will be noted that the greatest increase in terms of percentage was found to be for the sample representing the tenderloin muscle with an increase of 80.8%. The smallest increase was found to be 38.0% for the neck sample. The largest percentage increase in diameter of fiber between any 2 age groups was that between the 500-pound steer calf and 900-pound steer groups. Take, for example, the tenderloin muscle; from veal to steer calves the increase was 19.2% and from veal calves to 900-pound steers, 59.8%. Likewise, the difference between veal calves and heifers was 73.6%, and for cows, 80.8%. Similar results are shown for the remaining samples.

Likewise, there was little difference in the percentage rate of growth in fiber diameter for the 3 large muscles of the round. Again, the largest difference was found to be between 500-pound steer calves and the 900-pound steers. These results add confirmation to the premise that the greatest change in fiber diameter, as previously stated, was due to the rapid growth during this period of animal life.

TABLE 3

Analysis of variance of the tenderizing effect due to fiber size on beef animals of different ages and sexes

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Animal groups	6	25,672.51	4278.75*
Samples	8	16,080.27	2010.03*
Interaction	48	5,855.23	121.98†
Error	405	18,966.47	46.83
Total	467	66,574.48	

* Very highly significant
† Significant

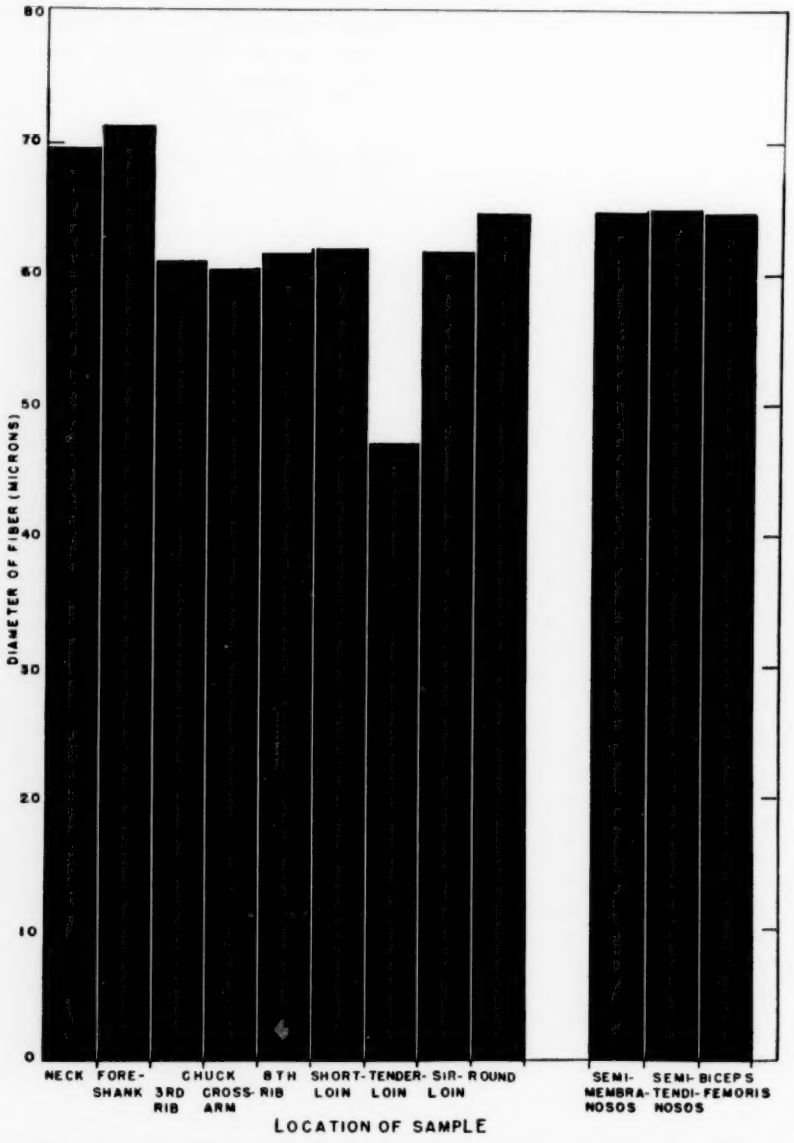

Figure 3. Relationship between fiber diameter of 9 muscles of beef and 3 muscles of round from cows.

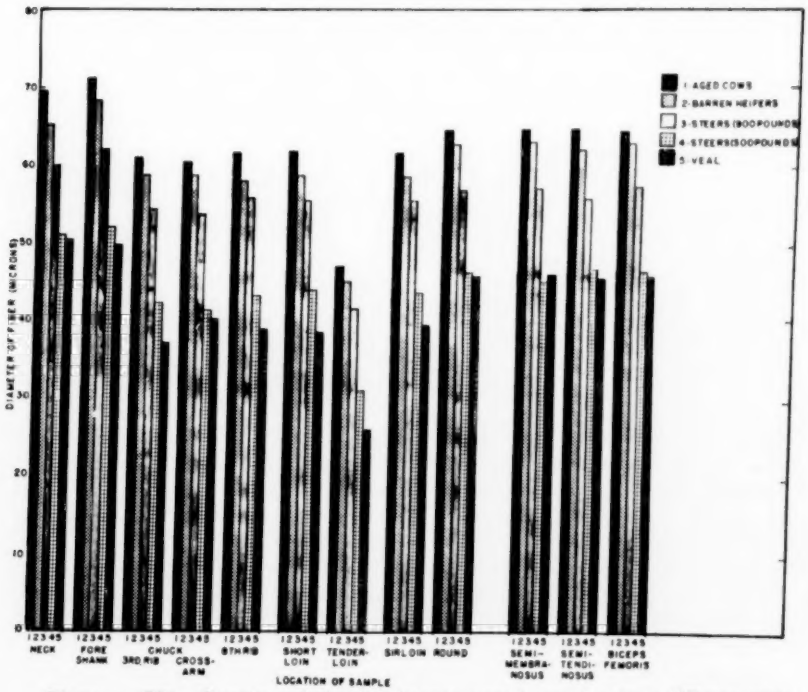

Figure 4. Fiber diameter relationship between muscle samples from different ages and sex.

An analysis of variance was made of the data. As shown in Table 3, the differences between animal groups and between muscle samples were both very highly significant, whereas the interaction of animals and samples was only significant.

One of the objects of this study was to determine whether there was a relationship between diameter of fiber and tenderness of the heated sample. To study this relationship, correlation coefficients between diameter of fiber and values for resistance to shearing were calculated, Snedecor *(14)*. The results are shown in Table 4. It will be noted that the correlation coefficients varied from +0.75 for the 52 neck samples to +0.31 for the 52 foreshank samples. All of the "r" values were very highly significant except the one for the foreshank which was only significant at the 5% level. Therefore, as fiber diameter increased, resistance to shearing increased. Further examination of Table 4 shows that those muscles earlier referred to as "working" muscles had higher correlation coefficients than those classified as "less worked," except in the case of the foreshank.

It may be noted that when all samples were considered as one population, the correlation was +0.64, which is highly significant at the 0.001 level, Table 4. However, as shown by the scatter diagram, Figure 4, the relationship is curvilinear. By using the simple parabola the correlation

TABLE 4

Coefficient of correlation of tenderness (resistance to shearing) and diameter of fiber for each of the muscle samples studied

Muscle Sample	Number of Samples	Correlation Coefficient "r"	Significance [a]
Neck	52	+0.75	$P < .001$
Foreshank	52	+0.31	$P < .05$
Chuck, 3rd rib	52	+0.60	$P < .001$
Chuck, Cross-arm	52	+0.68	$P < .001$
Eighth rib	52	+0.67	$P < .001$
Shortloin	52	+0.51	$P < .001$
Tenderloin	52	+0.54	$P < .001$
Sirloin	52	+0.64	$P < .001$
Round	52	+0.72	$P < .001$
All samples	468	+0.64	$P < .001$

[a] $P < .001$—Very highly significant; $P < .05$—Significant.

was found to be 0.83. The standard error of estimate was 5.26. The regression line, or curve of average relationship, shown in Figure 4 was that of a normal growth curve: $Ye = a + b \log x$, Ezekiel *(3)*.

To determine if diameter of fiber and resistance to shearing for samples representing any one age group were more closely related than those of another age, coefficients of correlation between the samples in each age group were determined. Table 5 shows that tenderness and fiber diameter in the mature animals were more closely associated than in the younger immature cattle. The coefficient of correlation in the case of cows was +0.77 and for veal calves +0.50. The intermediate age groups had intermediate "r" values. The coefficients for all age groups were very highly significant.

TABLE 5

Coefficient of correlation of tenderness (resistance to shearing) and diameter of fibers for nine samples in each age group

Animals Used	Number of samples	Correlation Coefficient "r"	Significance [b]
Cows	72	+0.77	$P < .001$
Heifers, barren	72	+0.61	$P < .001$
Steers—900 pounds	225	+0.63	$P < .001$
Steer calves—500 pounds	72	+0.58	$P < .001$
Veal calves	27	+0.50	$P < .001$

[b] $P < .001$—Very highly significant.

SUMMARY AND CONCLUSIONS

Studies were made to determine the relationship to tenderness of fiber diameter in beef muscles that varied widely in tenderness. Nine samples of muscle from each of 52 beef animals were used. The animals varied from 10-week-old veal calves to 9-year-old cows. The carcasses were aged approximately 14 days at 0.6 to 1.7°C. (33 to 35°F.), cut into their primal cuts and from these the 9 samples were prepared. The samples were weighed, heated to an internal temperature of 60°C.(140°F.), and tested objectively for tenderness. The histological sections were fixed in 10% formalin solu-

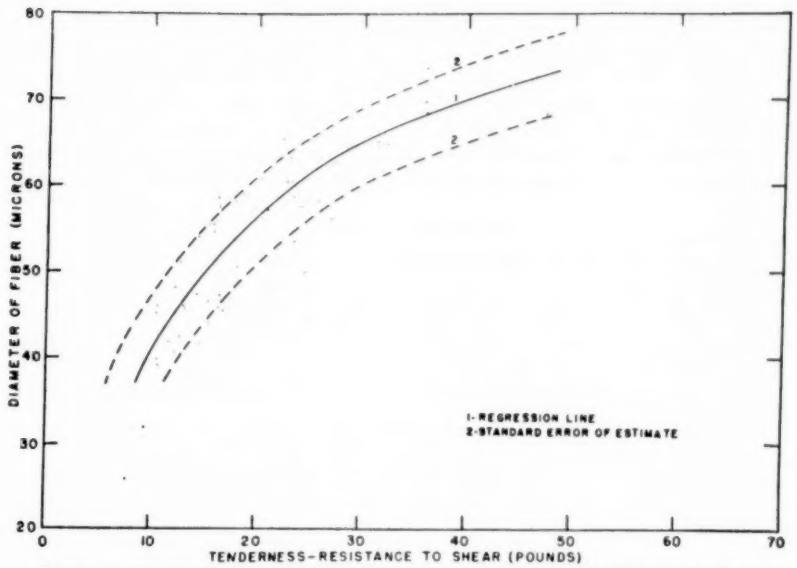

Figure 5. Scatter diagram of averages of fiber diameter and tenderness (resistance to shearing) of all muscles with curves of average relationships and standard errors of estimate.

tion for 5 to 8 days, after which ½ of each sample was embedded in paraffin and the other half used for fiber diameter determinations.

The diameters of the fixed fibers from the 9 samples classified themselves into 4 general groups in increasing magnitude as follows: (a) tenderloin; (b) the two chuck samples, 8th rib, shortloin and loin end; (c) round; (d) neck and foreshank. There was little difference in diameter of fiber for the 3 large muscles of the round, the semimembranosus, semitendinosus and biceps femoris. With increasing age of the animal there was a consistent increase in average muscle fiber diameter for all samples studied.

The difference in diameter of fiber of the muscles from veal calves and 500-pound steer calves was not significant at the 5% level. However, the difference between steer calves and 900-pound steers was highly significant. Other highly significant differences were found between cows and 900-pound steers, cows and 500-pound steer calves, and heifers and 500-pound steer calves.

In terms of percentage the psoas major (tenderloin) muscle fibers increased in size the most from veal to cows, whereas, the serratus ventralis (neck) muscle increased the least. In general the less active muscle fibers increased in size more than those that were more active.

Coefficients of correlation of tenderness (resistance to shearing) and diameter of fiber for each muscle ranged from an "r" of +0.75 for the neck sample to +0.31 for the foreshank. All coefficients of correlation were very highly significant except that of the foreshank. The relationship between tenderness and fiber diameter for all of the samples was shown to be

curvilinear. The curvilinear correlation was 0.83. All coefficients of correlation involving samples from any one age group were very highly significant. They varied from a low of +0.50 for veal calves to a high of +0.77 for mature cows. The correlation for steer calves was +0.58, 900-pound steers +0.63 and barren heifers +0.61. Analysis of variance showed that fiber diameter and tenderness of the animals and samples was very highly significant but the interaction of the two was only significant.

LITERATURE CITED

1. Beard, F. J. A study of tough and tender beef. (Unpublished master's thesis, Iowa State College, 1924).
2. Brady, D. E. A study of the factors influencing tenderness and texture of beef. *Proc. 30th Ann. Meeting Am. Soc. Anim. Prod.* (1937).
3. Ezekiel, M. *Methods of Correlation Analysis.* 1941, 2d ed., 5th printing, John Wiley & Sons, Inc., New York, N. Y.
4. Fisher, R. A. *Statistical Methods for Research Workers.* 1934, 5th ed., Rev. Oliver and Boyd, London.
5. Foster, M. T. Carcass comparison between a year-old steer and one nine years old. Missouri Agr. Experiment Sta. *Research Bull.* 117 (1926).
6. Hammond, J. *Growth and the Development of Mutton Qualities in the Sheep.* 1932, Oliver and Boyd, London.
7. Helser, M. D., Nelson, P., and Lowe, B. Influence of animal age upon quality and palatability of beef. I. Carcass yields and quality of meat cuts. II. The roast beef preparation, quality and palatability. Iowa Agr. Exp. Sta. Bull. 272 (1930).
8. Hiner, R. L., and Hankins, O. G. The tenderness of beef in relation to different muscles and ages in the animal. *J. An. Sci.*, **9**, 347-353 (1950).
9. Hirzel, R. Factors affecting quality in mutton and beef with special reference to the proportions of muscle, fat and bone. *Onderstepoort J. Vet. Sci. and Anim. Indus.*, Union of South Africa, **12**, 379-550 (1939).
10. Lehmann, K. B. Studien über die Zahigkeit des Fleisches und ihre Ursachen. *Arch. Hyg.*, **63**: 134-179, *Exp. Sta. Rec.*, **19**: 1161 (Abstract) (1907).
11. Moran, T., and Smith, E. C. Post-mortem changes in animal tissues, the conditioning or ripening of beef. (Brit.) Food Invest. Bd. *Special Report No. 36*, H. M. Stationery Off., London (1929).
12. Ramsbottom, J. M., Strandine, E. J., and Koonz, C. H. Comparative tenderness of representative beef muscles. *Food Research*, **10**, 497-509 (1945).
13. Robertson, D. D., and Baker, D. D. Histological differences in the muscles of full, half and rough fed steers. Missouri Agr. Exp. Sta. *Bull.* 200 (1933).
14. Snedecor, G. W. *Statistical Methods*, 1940, 3rd ed. The Collegiate Press, Inc., Ames, Iowa.

THE AMINO ACID COMPOSITION OF CHLORELLA[a]

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The potential importance of algae as food has stimulated numerous investigations on the large-scale production of *Chlorella pyrenoidosa*.[d] This prospect is attractive particularly because the fat and protein content of Chlorella vary widely depending upon the composition of the culture medium and other factors *(13)*. In the present experiments the amino acid composition of 2 species of Chlorella has been determined as an index of the nutritional quality of algal proteins.

EXPERIMENTAL[e]

Chlorella pyrenoidosa and *Chlorella vulgaris* (Emerson strain) were cultured aseptically in Hoagland and Arnon's *(9)* medium contained in 4-l. conical flasks, each fitted with a sealed in aeration tube. The flasks were aerated at the rate of 300 ml. of air per minute for 30 days in an unheated (temperature, 12°C. to 25°C.) glass house under natural conditions of illumination.

The cells, harvested by centrifugation, were extracted in a Soxhlet apparatus for 8 hours with acetone and then 8 hours with diethyl ether. The solid material in the extract represented about 9% (dry weight) of the cells (about 6.5 g. of dry cells per liter of medium). The solvent-extracted cells were extracted twice with cold and then once with hot trichloroacetic acid to remove as much non-protein material as possible.

For the amino acid assays 2.0 g. of dry product were heated for 18 hours with 20 ml. of 6 *N* HCl in a sealed pyrex tube in an autoclave at 18 lbs. pressure. Samples (0.25 g.) for the determination of tryptophan were heated under the same conditions with 10 ml. of 3 *N* NaOH. Each hydrolysate was diluted to volume in a volumetric flask and employed as stock solution in the assays. The amino acids were determined microbiologically by the following methods: alanine, Sauberlich and Baumann *(11)*, proline, Dunn *et al. (5)*, and the remaining amino acids, Dunn *et al. (3)*.

RESULTS AND DISCUSSION

The percentages of 17 amino acids in the crude protein (moisture-, ash-, and lipid-free material from chloroacetic acid-extracted cells) from two strains of Chlorella are given in Table 1. The probable maximum errors of these values are approximately 5% of the mean values. The values reported by Combs *(2)* and those of the authors corresponding, generally are of the same order of magnitude although not quantitatively in good agreement. Differences in strains, culture conditions, cell treatment or, possibly, microbiological assay procedures may account for the disagreement.

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[d] See Cook *(1)* and Myers *et al. (10)* for reviews of the earlier literature.

[e] Cultures of the Chlorella strains were kindly supplied by Dr. D. Appleman, Department of Irrigation and Soils, University of California, Los Angeles.

TABLE 1

Amino acid composition of chlorella [f]

Amino acid	*Chlorella pyrenoidosa*			*Chlorella vulgaris*
	Authors	Combs (8)		Emerson [j]
	g	h	i	Authors
Alanine	5.59	.....	.....	5.24
Arginine	6.07	4.71	5.97	3.82
Aspartic acid	7.91	.....	.....	6.32
Cystine	0.83	.....	.....	0.61
Glutamic acid	8.65	.....	.....	7.60
Glycine	4.25	.....	5.50	4.80
Histidine	2.00	1.44	1.62	1.42
Isoleucine	5.31	4.00	4.23	5.93
Leucine	8.71	8.66	4.98	8.37
Lysine	5.13	4.71	6.08	2.23 [k]
Methionine	0.48	0.82	1.45	0.96
Phenylalanine	4.97	4.14	5.35	11.58
Proline	3.99	.....	.....	3.48
Threonine	4.32	4.85	4.78	2.26
Tryptophan	1.22	1.83	1.05	1.11
Tyrosine	1.77	.....	.....	3.24
Valine	5.72	5.65	6.67	5.29
Total	76.9	.....	.....	74.2

[f] Values given as per cent amino acid of protein (N × 6.25) in extracted, dried cells.
[g] Nitrogen, 6.93%; moisture, 8.27%; ash, 1.03%.
[h] Nitrogen, 7.0(44.0/6.25)%. Microbiological assay by Food Research Laboratories of cells grown by Arthur D. Little, Inc.
[i] Nitrogen, 6.4(40.0/6.25)%. Microbiological assay by Merck and Company of cells grown by Spoehr and Milner (3).
[j] Nitrogen, 6.54%; moisture, 10.15%; ash, 0.08%.
[k] Value seems to be incorrect.

From tests on the food potentialities of dried (presumably solvent-extracted) *Chlorella pyrenoidosa*, Combs (2) concluded that this alga supplied important quantities of carotene and certain B-complex vitamins significant in the nutrition of the chick. On the other hand, the inclusion of 10% dried cells in the diet in place of an equal quantity of soybean oil meal resulted in an average body weight which after 4 weeks was lower than that on the complete broiler mash or the basal ration supplemented with 0.1% DL-methionine. This experiment was designed to test what was presumed to be a low level of methionine in Chlorella protein compared to soybean. It was suggested that the growth-depressing action of the dried Chlorella resulted from lowered food consumption and that the latter was due to an impacted beak condition caused by the hygroscopic nature of the material.

These tests seem inadequate and should be repeated. Data inconsistent with the conclusions of Combs (2) have been reported recently by Fowden (7, 8). As shown in Table 2, the methionine content of *Chlorella vulgaris* protein, containing about 75% of the total nitrogen of the cells, is approximately twice that of soybean protein. Although grossly deficient in cystine and moderately so in methionine and histidine, apparently Chlorella protein contains adequate quantities of other amino acids. It seems a logical assumption, therefore, that Chlorella protein, and possibly cells, may serve

TABLE 2

Amino acid composition of chlorella and other proteins [1]

Amino acid	*Chlorella vulgaris* [m] Fowden (*9, 10*)	Soybean Dunn *et al.* (*11*)	Whole hen's egg Dunn *et al.* [n]
Alanine	7.8	.....	.....
Arginine	7.9	7.0	5.7
Aspartic acid	9.7	8.8	10.5
Cystine	0.27	1.2	2.3 [o]
Glutamic acid	13.1	20.5	12.6
Glycine	5.4	4.2	3.0
Histidine	2.0	2.5	2.3
Isoleucine	5.2	6.3	6.8
Leucine	9.1	7.9	9.2
Lysine	8.4	6.6	7.4
Methionine	2.4	1.2	3.0
Phenylalanine	5.2	5.3	5.1
Proline	6.0	4.8	.....
Serine	4.0	.....	8.4 [p]
Threonine	3.9	4.0	4.1
Tryptophan	2.4	1.1	1.0
Tyrosine	5.8	4.1	3.2
Valine	7.3	6.7	6.9
Total	115.6	102.2	91.5

[1] Values given as per cent amino acid of protein (N × 6.25).
[m] Calculated from the relation: g. amino acid N/100 g. protein N × 16/100 × molecular weight of amino acid/g. of amino acid per mole of amino acid.
[n] Dunn, M. S., Shankman, S., Camien, M. N., and Block H., unpublished data.
[o] Value given by Evans *et al.* (*12*).
[p] Value given by Schormüller and Walter (*13*).

satisfactorily as a source of amino acids in animal feeding and, if supplemented with cystine, methionine, and histidine, would be equivalent to other proteins of high nutritional quality. It is the authors' intention to acquire experimental evidence bearing on this problem.

SUMMARY

The percentages of 17 amino acids in the crude protein from 2 strains of Chlorella have been determined by microbiological assay procedures. It has been concluded tentatively that Chlorella protein, and possibly cells, may serve satisfactorily as a source of amino acids in animal feeding if supplemented with cystine, methionine, and histidine.

LITERATURE CITED

1. Cook, P. M. The Phycological Society of America, Annual Meeting, New York City, Dec. 28, 1949.
2. Combs, G. F. *Science*, **116**, 453 (1952).
3. Dunn, M. S., Camien, M. N., Malin, R. B., Murphy, E. A., and Reiner, P. J. Univ. Calif. *Publ. Physiol.*, **8**, 293 (1949).
4. Dunn, M. S., Camien, M. N., Shankman, S., and Rockland,, L. B. *The Determination of Amino Acids in Soybean Protein by Microbiological Procedures*, Report to Committee on Protein Foods, National Research Council, May 18, 1945.
5. Dunn, M. S., McClure, L. E., and Merrifield, R. B. *J. Biol. Chem.*, **179**, 11 (1949).
6. Evans, R. J., Davidson, J. A., and Butts, H. A. *Poultry Sci.*, **29**, 104 (1950).

7. FOWDEN, L. *Biochem. J.*, **50**, 355 (1951).
8. FOWDEN, L. *Biochem. J.*, **52**, 310 (1952).
9. HOAGLAND, D. R., AND ARNON, D. I., Calif. Agr. Exp. Sta. *Bull. 347*, 31 (1950).
10. MYERS, J., PHILLIPS, J. N., JR., AND GRAHAM, J. R. *Plant Physiol.*, **26**, 539 (1951).
11. SAUBERLICH, H. E., AND BAUMANN, C. A. *J. Biol. Chem.*, **166**, 417 (1946).
12. SCHORMÜLLER, J., AND WALTER, J. *Deut. Lebens.-Rundschau*, **47**, 265 (1951).
13. SPOEHR, H. A., AND MILNER, H. W. *Plant Physiol.*, **24**, 120 (1949).

ORGANOLEPTIC RATINGS OF WINES ESTIMATED FROM ANALYTICAL DATA

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Wines are complex from the standpoint of their chemical and physical properties. Small changes in such properties may result in relatively large changes in organoleptic response by some tasters; large changes, as shown by analytical results, may be entirely overlooked organoleptically. To find and develop tasters with sufficient technical background and organoleptic discrimination to insure uniform and consistent ratings of diverse types of wines has proved most difficult.

The purpose of this study is to present detailed organoleptic and analytical data on 2 types of wines (Cabernet and Riesling) for 5 highly trained tasters. The possibility of replacing organoleptic ratings of highly trained experts by routine analyses by laboratory technicians and statistically derived estimating equations is also examined.

It will be worth while to have in mind a few of the primary facts revealed in this study. Only a few analytic characteristics discriminate between Cabernets; more, but not all, of the characteristics considered distinguish the Rieslings. Some analytical data discriminate between tasters although they may, or may not, help indicate differences between wines within a type. Certain analytical variables have been selected as a basis for predicating organoleptic scores. In some cases the percentage variation taken care of by the estimating equations is high enough to be definitely encouraging. Better results are obtained for Riesling than for Cabernet wines. Some tasters even among those highly trained are more consistent than others and have much greater sensitivity to varying chemical and physical properties of the wines. These facts will be made evident in greater detail in the course of this article.

REVIEW OF THE PROBLEM

Many attempts have been made to replace organoleptic ratings of foods and beverages by observation of certain of their chemical and physical characteristics. In some instances a measure of success has been attained. Blanchard and Maxwell *(2)*, for example, found correlation coefficients of 0.73 and 0.74 between sugar content and tasting scores of peas picked at different stages of maturity. Although such correlations are not always possible, Boggs and Hanson *(3)*, Wagner *(6)* give many successful correlations.

Most investigators end up by accepting organoleptic testing as the final authority despite the fact that the establishment and application of organoleptic standards by different people is extremely difficult. Because of this difficulty and the general complexity of the whole subject of organoleptic testing, there has been a tendency to accept certain individuals as "authorities" without too careful an inquiry into the consistency of their ratings and their ability to detect and evaluate undoubted organoleptic differences.

Recently food technologists have come to depend on trained panels or on consumer acceptance tests. Trained panels have this advantage—they can be trained to detect certain differences, and if the proper methods are used the results can be analyzed for

statistical significance. Consumer preference tests can be used to predict certain levels of consumer acceptance and may be utilized profitably by those catering to well-defined groups of consumers, but an adequate methodology for this latter type of testing for application to foods of varying kinds, prices, and qualities with due consideration for the immense range of consumer groups (racial, economic, religious, urban and rural, etc.) has yet to be developed.

With reference to wine, the need for a method for accurately predicting quality has been stressed by Palieri and Garoglio *(5)* and Klenk *(4)*. Palieri and Garoglio developed a score card which when multiplied by the nominal market value was to be used as the actual market price. Klenk, who also developed a score card, carefully annotated each part of it as to the plus or minus points to be awarded. The scores obtained were to be used along with must and wine analyses. No correlations were attempted, and the number of examples reported was very small.

EXPERIMENTAL

Organoleptic tests were made by 5 individuals on 13 Cabernet wines and 17 Riesling wines. The results together with corresponding observations on certain chemical and physical characteristics of the wines comprise the experimental data. Procedures were as follows:

The tasters were told the type of wine being tasted and were asked to score the wine. The taster had 4 or 5 wines to taste at a session. Tasters worked completely independently of each other. Cabernet wines were served at room temperature (70°F.), and Riesling wines at about 55°F. Tulip-shaped clear-glass wine glasses were used.

The score sheets used were those employed in this laboratory, see Amerine *(1a)*. All tasters were familiar with and had used the score card. Furthermore, all tasters were familiar with the 2 types of wines. However, no preliminary warm-up tasting or training period was employed.

The wines were commercial California Cabernet and Riesling table wines which had been submitted to the 1947 California State Fair for judging. They represented a cross section of the best and worst wines of these types as the over-all scores reveal. The range—64 to 85—is about the maximum normally obtained with this score card.

Immediately after the tastings the residual wine was analyzed by the usual methods of this laboratory, Amerine *(1b)*. The analyses are summarized in Table 1. The total and partial organoleptic scores are given in Tables 2 and 3.

Analysis of data. Analyses of variance were run on the total and partial organoleptic scores in order to determine the consistency of the ratings of the 5 tasters (Table 4). F values significant at the 5% level are indicated by one asterisk; at the 1% level by 2 asterisks.

Organoleptic ratings were given separately on some of the chemical and physical characteristics. In order to study the predictability of organoleptic ratings for these single factors by means of more objective determinations correlation coefficients were computed. Since it is not pertinent to the purpose of this study to consider these data as random samples from some hypothetical population and discuss their "significance" on this basis, these coefficients will be regarded as measures of the predictability of organoleptic scores in terms of objective measures. The correlations ranged from —0.82 to 0.80, and though many were insignificant some were high enough to be encouraging.

Even though the predictability of organoleptic ratings is low, in general, for one independent variable, it may be that using several characteristics objectively measured will give very good results. In order to help select good predictors the correlations between the various objectively determined characteristics of Table 1 were computed.

On the basis of the correlation coefficients and general intuitive considerations 5 variables were selected for Cabernets and 6 for Rieslings. Variables selected for Cabernets were alcohol, total acid, hydrogen ion concentration, tannin, and volatile acid. The variables selected for Rieslings were total acid, volatile acid, sulphur dioxide, aldehyde, hydrogen ion concentration, and color.

On the basis of the indicated variables in the order listed, linear estimating equations for total organoleptic scores for each taster and 2 combinations of tasters were computed. The coefficients varied markedly for the different tasters.

In order to study the efficacy of the proposed method of estimating total organoleptic ratings the observed and computed ratings are compared in Table 5. The correspondence

TABLE 1

Cabernet

	236	237	238	239	240	241	242	243	244	245	246	247	248
Balling	−1.1	−1.0	−1.5	−1.7	−1.6	−1.1	−1.6	−1.4	−1.9	−1.3	.....	−1.5	−1.5
Alcohol	12.1	11.5	13.8	12.2	12.2	12.7	13.1	13.3	12.8	13.5	13.3	12.9	12.8
Extract	3.1	3.0	3.3	2.8	2.8	3.4	3.0	3.2	2.3	3.3	2.3	2.6	2.9
pH	3.55	3.32	3.80	3.46	3.55	3.49	3.48	3.59	3.42	3.40	3.46	3.65	3.39
Total acid	0.71	0.82	0.64	0.65	0.72	0.81	0.69	0.58	0.59	0.71	0.57	0.62	0.73
Volatile acid	0.063	0.104	0.064	0.055	0.134	0.111	0.080	0.075	0.065	0.078	0.061	0.068	0.065
Sugar	0.16	0.145	0.290	0.12	0.10	0.13	0.14	0.335	0.14	0.11	0.06	0.165	0.165
Total SO_2	6	31	0.0	0.0	13	63	51	0.0	29	32	25	13	6
Aldehyde	55	40	79	18	25	48	42	27	.....	.....	.....	.....	.....
Tannin	0.359	0.326	0.323	0.240	0.257	0.462	0.325	0.154	0.163	0.344	0.231	0.282	0.265
Color	205	256	238	196	105	476	263	164	238	333	173	217	167
Iron	1.06	2.98	2.25	1.94	1.66	1.33	2.90	1.57	3.47	5.80	4.10	2.32	2.85
Copper	0.0	0.0	0.16	0.0	0.0	0.23	0.17	8.58	0.65	0.47	0.41	0.65	1.06
Total neutral esters	319	349	408	259	468	438	358	338	339	289	299	299	396
Volatile neutral esters	71	92	61	101	121	173	112	81	81	102	102	102	142
Glycerin	0.98	0.95	1.04	1.14	1.22	0.83	1.12	0.91	.....	0.80	1.19	0.96	.....
Ash, gr./l.	3.22	2.60	2.64	2.68	2.836	2.666	3.144	3.312	2.456	3.152	2.084	3.188	2.264
Alkalinity of ash, ml. 0.1 N/100 ml.	27.6	22.4	29.2	23.4	27.4	22.8	24.21	21.20	23.40	23.00	22.20	30.20	24.10
K_2SO_4, gr./l.	.....	2.0	1.21	0.77	0.87	1.13	1.26	1.78	0.95	1.49	0.74	1.06	1.21
Volatile acid corrected for SO_2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

Riesling

	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146
Balling	−2.0	−1.8	−1.9	−1.9	−1.9	−1.8	−2.0	−1.4	−2.1	−2.0	−1.9	−2.0	−2.2	−1.7	−2.2	−1.3	−1.7
Alcohol	11.4	11.4	12.6	11.5	11.2	12.0	12.7	11.4	12.9	12.3	11.2	12.2	12.0	11.8	12.7	10.2	12.3
Extract	2.0	2.3	2.4	2.2	2.2	2.3	2.5	2.7	2.5	2.4	2.1	2.4	2.0	2.6	2.3	2.4	2.6
pH	3.59	3.10	3.39	3.10	3.31	3.37	3.51	3.46	3.38	3.31	3.19	3.51	3.50	3.17	3.10	3.33	3.55
Total acid	0.46	0.64	0.62	0.67	0.56	0.55	0.60	0.75	0.58	0.56	0.64	0.53	0.44	0.68	0.59	0.68	0.61
Volatile acid	0.073	0.070	0.071	0.049	0.049	0.056	0.040	0.083	0.054	0.070	0.061	0.063	0.057	0.070	0.066	0.135	0.083
Sugar	0.090	0.125	0.150	0.090	0.140	0.085	0.220	0.165	0.175	0.130	0.135	0.145	0.175	0.080	0.165	0.055	0.100
Total SO_2	57	83	114	107	101	76	82	157	120	140	159	130	64	70	111	137	89
Aldehyde	69	77	88	100	58	40	113	45	80	31	88	100	58	98	51	132	48
Tannin	0.017	0.034	0.026	0.026	0.034	0.026	0.043	0.026	0.017	0.043	0.017	0.043	0.026	0.009	0.034	0.017	0.017
Color	22	25	27	10	19	14	16	17	12.5	15	12	18	17	14	12	16	28.5
Iron	3.3	3.2	3.9	2.4	0.52	0.0	1.1	4.3	.....	.....	.....	.....	.....	.....	.....	.....	.....
Copper	0.63	0.63	1.06	0.39	1.25	0.0	0.28	0.44	0.30	0.28	0.13	0.21	0.71	0.25	0.22	0.0	0.48
Total neutral esters	286	320	252	179	167	210	167	357	214	250	238	333	286	238	274	316	476
Volatile neutral esters	32	28	19	19	20	21	21	20	20	20	20	21	20	41	20	61	82
Glycerin	0.94	1.03	1.03	0.89	0.95	1.04	1.03	0.90	0.75	0.74	0.66	0.69	0.66	0.74	1.03	0.54	0.83
Ash, gr./l.	2.44	2.57	2.66	1.78	2.14	2.02	2.91	3.18	2.20*	2.37	2.18	2.67*	2.50	2.42	1.96*	2.75	3.412
Alkalinity of ash, ml. 0.1 N/100 ml.	23.2	13.2	19.5	14.6	19.9	18.7	24.4	28.4	20.95*	17.45	17.8	25.1*	20.5	17.0	11.9	20.5	28.9
K_2SO_4, gr./l.	0.750	1.52	1.20	0.698	0.780	0.695	1.03	0.984	0.753	1.10	0.844	0.915	1.05	1.19	1.03	1.19	1.31
Volatile acid corrected for SO_2	.....	.....	0.051	0.033	0.032	.....	.....	0.069	.....	0.043	0.043	.....	.....	.....	0.046	0.113	.....

* Duplicates.

TABLE 2

Total and partial organoleptic scores on certain characteristics for five tasters for 13 Cabernets

Wine no.	Taster	Appearance	Color	Aroma and bouquet	Vol. acid	Total acid	Sugar	Body	Taste	Tannin	General quality	Total score
236	A	10	10	9	10	6	8	5	9	6	2	75
236	B	8	10	8	10	8	6	3	8	5	4	70
236	C	8	9	11	9	6	7	3	9	6	2	70
236	D	8	8	11	10	6	6	5	10	8	3	75
236	E	10	8	7	10	7	8	5	8	9	0	72
237	A	10	10	8	9	7	8	5	7	6	0	70
237	B	8	10	10	10	6	8	3	10	7	4	76
237	C	10	10	10	8	7	8	4	10	7	3	77
237	D	9	8	9	10	7	6	5	9	8	2	73
237	E	10	9	11	10	7	8	4	8	8	4	79
238	A	10	10	12	10	6	8	5	9	7	5	82
238	B	8	8	8	10	8	8	3	8	5	3	69
238	C	10	10	10	10	8	8	5	10	6	2	79
238	D	9	9	13	10	6	5	5	10	8	4	79
238	E	10	5	5	10	6	8	5	6	7	2	64
239	A	10	9	8	10	7	8	4	8	6	0	70
239	B	8	9	10	10	8	8	3	9	7	4	76
239	C	9	9	8	9	7	7	3	8	6	3	70
239	D	9	8	10	10	7	6	5	8	6	2	71
239	E	10	8	10	10	7	8	5	8	7	4	77
240	A	10	9	8	7	7	8	4	8	6	2	69
240	B	8	10	12	7	6	8	6	10	7	4	78
240	C	9	10	11	10	7	8	4	8	8	2	77
240	D	9	9	10	10	6	6	5	8	6	0	69
240	E	10	8	8	5	8	8	5	8	8	2	70
241	A	10	10	4	10	8	8	5	7	6	−8	60
241	B	8	10	7	10	4	8	4	7	5	3	66
241	C	9	9	6	10	6	7	5	9	8	0	69
241	D	9	9	5	8	5	5	4	5	5	0	55
241	E	10	10	9	10	6	8	6	3	7	0	69
242	A	10	10	9	10	7	8	5	8	5	0	72
242	B	8	10	8	10	8	8	4	7	6	4	73
242	C	9	9	9	9	5	7	4	9	6	3	70
242	D	9	9	8	10	5	6	4	8	6	2	67
242	E	10	8	7	10	7	8	5	7	6	4	72
243	A	10	10	12	9	8	8	4	9	8	3	81
243	B	8	8	7	10	4	8	6	7	3	3	64
243	C	10	8	8	10	5	7	4	10	9	2	73
243	D	10	10	12	8	5	6	5	10	6	4	76
243	E	10	7	10	10	7	8	5	12	10	4	83
244	A	9	10	10	10	6	7	5	8	7	5	77
244	B	8	8	10	10	8	8	3	10	8	4	77
244	C	9	10	10	10	7	8	4	9	8	3	78
244	D	8	8	11	10	6	6	5	12	8	4	78
244	E	10	8	12	10	7	7	5	9	7	4	79
245	A	10	9	12	8	8	8	5	8	7	6	82
245	B	8	10	12	10	6	8	5	11	7	5	82
245	C	10	10	10	9	7	8	5	9	6	4	78
245	D	9	8	13	10	6	6	5	13	8	5	83
245	E	10	7	12	10	7	8	6	8	8	5	81
246	A	10	9	8	10	8	8	4	8	8	0	73
246	B	10	7	8	10	6	8	4	8	8	4	73
246	C	10	9	9	10	7	8	4	10	7	3	77
246	D	9	7	8	8	5	6	4	12	8	3	70
246	E	10	8	6	10	6	8	4	6	8	4	70
247	A	10	9	12	10	8	8	4	9	8	3	81
247	B	9	8	8	10	6	8	3	7	8	4	71
247	C	10	10	10	10	7	8	4	11	8	4	82
247	D	9	8	11	10	6	6	5	10	8	3	76
247	E	10	8	10	10	7	8	6	7	7	5	78
248	A	10	9	11	8	8	8	4	8	7	3	76
248	B	10	8	9	10	6	8	4	9	8	4	76
248	C	9	9	12	10	7	8	5	9	10	4	83
248	D	9	9	8	10	6	6	5	8	8	2	71
248	E	10	8	9	10	7	8	4	7	8	4	75

TABLE 3

Total and partial organoleptic scores on certain characteristics for five tasters for 17 Rieslings

Wine no.	Taster	Appearance	Color	Aroma and bouquet	Vol. acid	Total acid	Sugar	Body	Taste	Tannin	General quality	Total score
130	A	9	9	10	10	6	8	5	10	8	3	78
130	B	10	9	8	10	8	5	4	8	9	4	75
130	C	10	8	10	10	6	8	4	11	9	3	79
130	D	9	9	11	10	7	5	5	11	9	3	79
130	E	10	8	8	8	6	7	5	9	9	2	72
131	A	9	6	8	8	6	8	6	8	6	3	68
131	B	8	8	9	10	6	8	6	8	8	3	74
131	C	10	7	8	10	7	8	3	9	8	1	71
131	D	8	7	8	9	5	6	4	9	8	2	66
131	E	10	5	5	6	4	8	6	5	9	0	58
132	A	9	7	10	9	7	8	5	10	8	4	77
132	B	8	8	7	10	6	8	6	9	6	3	71
132	C	10	8	9	10	6	8	3	8	9	1	72
132	D	9	8	9	10	5	6	4	10	8	3	72
132	E	10	6	5	10	7	8	5	8	9	3	71
133	A	10	10	10	10	8	8	4	11	7	2	80
133	B	8	9	12	10	5	8	5	9	10	5	81
133	C	10	10	9	10	7	8	3	10	9	2	78
133	D	9	9	9	10	6	6	5	9	8	2	73
133	E	10	9	9	10	8	8	5	8	8	5	80
134	A	7	6	10	10	6	8	4	9	8	3	71
134	B	6	7	10	10	6	8	6	9	9	3	74
134	C	8	9	10	10	6	8	4	9	9	2	75
134	D	9	9	10	10	5	5	5	10	8	3	74
134	E	10	7	8	10	6	8	5	8	7	2	71
135	A	9	8	12	10	7	8	6	11	8	5	85
135	B	8	9	11	10	7	8	5	10	10	5	83
135	C	10	10	12	10	6	8	5	10	8	5	84
135	D	9	9	6	10	6	6	5	9	8	2	70
135	E	10	10	11	10	7	8	6	8	7	4	81
136	A	9	9	10	10	7	8	4	10	8	3	78
136	B	10	7	7	10	8	8	4	8	9	3	74
136	C	10	9	11	10	7	8	4	11	8	3	81
136	D	9	9	9	10	6	6	5	10	8	2	74
136	E	10	10	7	10	7	8	5	7	7	1	72
137	A	8	8	8	8	8	8	5	9	7	2	71
137	B	10	8	9	10	8	7	4	9	8	3	76
137	C	10	9	7	10	6	8	3	9	8	2	72
137	D	9	9	8	10	7	6	4	10	8	2	73
137	E	10	10	8	10	6	7	5	7	7	2	72
138	A	10	10	10	10	6	8	5	10	9	5	83
138	B	10	10	11	10	6	8	6	10	7	5	83
138	C	10	10	12	10	6	8	5	10	8	4	83
138	D	9	9	12	10	7	6	5	12	8	5	83
138	E	10	10	10	10	6	8	5	10	8	6	83
139	A	9	10	9	8	5	8	5	8	10	1	73
139	B	9	10	13	10	5	8	6	11	8	6	86
139	C	10	10	10	10	6	8	4	8	8	2	76
139	D	9	9	10	10	5	6	5	9	7	3	73
139	E	8	10	9	10	7	8	5	8	8	5	78
140	A	10	10	8	10	7	8	3	8	9	5	78
140	B	8	10	12	10	7	8	5	9	7	5	81
140	C	10	9	9	10	6	8	3	7	8	2	72
140	D	9	9	10	10	5	6	5	10	7	3	74
140	E	10	8	7	10	7	8	4	7	7	4	72
141	A	10	10	9	10	5	8	3	8	9	3	75
141	B	9	10	8	10	7	8	5	10	7	4	78
141	C	10	10	11	10	5	8	5	10	8	3	80
141	D	9	9	8	9	5	6	5	8	7	2	68
141	E	8	9	8	10	6	8	5	6	7	3	70
142	A	10	10	10	10	5	8	5	7	10	4	79
142	B	10	10	8	10	8	8	4	9	7	3	77
142	C	10	10	10	10	5	8	4	9	9	3	78
142	D	9	8	9	10	4	6	5	10	7	2	70
142	E	10	10	6	10	6	7	5	7	8	3	72
143	A	9	10	8	10	6	8	4	7	8	0	70
143	B	9	6	7	10	8	8	4	7	7	2	68
143	C	10	10	9	10	7	8	3	8	8	2	75
143	D	9	9	8	10	4	6	5	8	7	2	68
143	E	9	10	6	10	6	8	6	6	8	3	72
144	A	10	10	9	10	7	8	3	7	8	1	73
144	B	8	8	7	10	5	8	4	7	5	2	64
144	C	10	10	10	10	7	8	4	9	9	3	80
144	D	9	9	8	10	4	6	5	9	7	2	69
144	E	10	10	9	10	7	8	5	8	7	3	77
145	A	10	9	8	8	6	8	3	8	9	2	71
145	B	10	6	0	0	0	8	4	0	0	0	28
145	C	10	10	8	7	6	8	4	8	9	1	71
145	D	9	8	9	10	4	6	5	9	7	2	69
145	E	10	8	6	7	6	8	5	6	7	1	64
146	A	9	7	7	9	7	8	6	7	8	0	68
146	B	8	0	5	10	4	8	4	0	5	0	44
146	C	10	8	9	8	5	8	4	7	9	1	69
146	D	9	7	8	10	3	6	5	8	7	2	65
146	E	10	7	9	8	6	8	6	6	7	2	69

TABLE 4

Analysis of variance for total and partial scores for five tasters for 13 Cabernets and 17 Rieslings

Characteristics	F Between wines	F Between tasters
Cabernet		
Appearance	2.04*	27.54**
Color	1.33	8.68**
Aroma and bouquet	3.27**	.76
Vol. acid	2.62*	.44
Total acid	.94	3.86**
Sugar	1.18	61.24**
Body	1.10	4.74**
Taste	2.30*	4.74**
Tannin	1.91	2.48
General quality	4.15**	3.76**
Total	4.34**	.96
Riesling		
Appearance	1.94*	8.42**
Color	4.99**	2.73*
Aroma and bouquet	2.86**	3.55*
Vol. acid	32.71**	7.20**
Total acid	1.90*	3.27*
Sugar	2.61**	111.28**
Body	1.46	8.45**
Taste	3.76**	6.50**
Tannin	0.96	2.89*
General quality	4.79**	2.10
Total	4.12**	1.90

* Significant at the 5% level.
** Significant at the 1% level.

between observed and computed values is summarized by the multiple correlation coefficients squared which indicate the proportion of the variation as measured by the squares of deviations from the expected, accounted for by the estimating equations. These values of the squared multiple correlation coefficients are given in Table 6.

DISCUSSION

A major purpose of this paper is to indicate problems involved in relating organoleptic scores to analytical data. In the first place it appears that different, rather closely related products (Cabernets and Rieslings) can be examined organoleptically with quite different results. For Cabernets differences between wines were found for appearance, aroma and bouquet, volatile acid, taste, general quality, and total. The differences between tasters were indicated at a higher level of significance and for more characteristics. Differences between tasters were indicated by appearance, color, total acid, sugar, body, taste, and general quality. These results were obtained even with highly trained tasters who had an extensive acquaintance with both Cabernet and Riesling wines. The tasters had had experience in wine tasting for periods of from 5 to 15 years; 3 of them had participated in the wine judgings at the California State Fair.

Differences between the Rieslings were indicated for more characteristics and at higher levels of significance than for Cabernets. The characteristics

TABLE 5

Estimated and actual scores for 13 Cabernet and 17 Riesling wines

Wine No.	A		B		C		D		E		Average of 3 tasters A, C, E		Average of 5 tasters	
	Actual score	Est. score	Actual score	Est. score	Actual score	Est. score	Actual score	Est. score	Actual score	Est. score	Actual score	Est. score	Actual score	Est. score
							Cabernet							
236	75	71.5	70	70.7	70	72.0	75	70.8	72	71.7	72.3	71.7	72.4	**71.6**
237	70	66.9	76	77.1	77	74.6	73	68.3	79	77.5	75.3	73.0	75.0	**73.2**
238	82	83.0	69	66.8	79	78.3	79	77.3	64	68.0	75.0	76.5	74.6	**74.8**
239	70	75.2	76	73.8	70	74.9	71	75.2	77	77.3	72.3	75.8	72.8	75.0
240	69	67.8	78	72.8	77	75.6	69	67.8	70	73.3	72.0	71.6	72.6	71.0
241	60	65.9	66	72.3	69	71.8	55	63.3	69	68.8	66.0	67.8	65.8	69.5
242	72	73.5	73	73.4	70	74.9	67	70.8	72	73.6	71.3	74.0	70.8	73.6
243	81	81.2	64	73.1	73	79.6	76	78.3	83	77.1	79.0	79.3	75.4	77.5
244	77	77.0	77	75.6	78	76.8	78	76.1	79	79.8	78.0	76.8	77.8	75.7
245	82	74.8	82	75.5	78	76.4	83	71.5	81	75.3	80.3	75.5	81.2	75.2
246	73	75.2	73	73.8	77	73.9	70	72.8	70	76.2	73.3	75.1	72.6	73.5
247	81	76.1	71	69.4	82	74.4	76	73.4	78	71.4	80.3	73.9	77.6	**73.0**
248	76	78.8	76	76.6	83	79.7	71	77.4	75	79.0	78.0	79.2	76.2	**78.1**
							Riesling							
130	78	76.9	75	64.8	79	78.8	79	71.7	72	71.0	76.3	75.5	76.6	**72.2**
131	68	68.3	74	65.0	71	69.9	66	66.0	58	63.6	65.7	67.3	67.4	66.1
132	77	70.7	71	66.5	72	70.3	72	69.6	71	64.1	73.3	68.4	72.6	67.8
133	80	77.3	81	80.5	78	78.6	73	72.6	80	75.8	79.3	77.2	78.4	77.4
134	71	75.7	74	83.2	75	76.8	74	72.0	71	73.4	72.3	75.3	73.0	76.1
135	85	77.8	83	79.4	84	80.6	70	72.5	81	78.9	83.3	79.1	80.6	77.6
136	78	80.4	74	80.4	81	80.7	74	75.4	72	75.0	77.0	78.7	75.8	78.3
137	71	71.9	76	68.4	72	72.2	73	72.2	72	75.8	71.7	73.3	72.8	71.4
138	83	79.3	83	81.8	83	79.9	83	75.1	83	77.5	83.0	78.9	83.0	78.9
139	73	75.3	86	79.8	76	76.5	73	72.7	78	76.9	75.7	76.3	77.2	76.2
140	78	76.5	81	81.2	72	76.2	74	73.9	72	74.9	74.0	75.9	75.4	**78.3**
141	75	78.6	78	75.8	80	77.8	68	75.2	70	72.1	75.0	76.1	74.2	76.0
142	79	79.8	77	77.2	78	82.0	70	73.5	72	76.0	76.3	79.2	75.2	77.6
143	70	74.1	68	63.4	75	76.6	68	69.4	72	72.0	72.3	74.2	70.6	**71.0**
144	73	75.2	64	77.3	80	77.4	69	70.8	77	76.0	76.7	76.2	72.6	**75.6**
145	71	70.4	28	32.2	71	70.8	69	67.9	64	64.3	68.7	68.5	60.6	**61.3**
146	68	69.6	44	60.0	69	70.7	65	68.5	69	66.7	68.7	69.0	60.3	**65.9**

TABLE 6

Multiple correlation coefficients squared for five tasters and two types of wines (Cabernet and Riesling)

Taster	Cabernet	Riesling
A	0.64	0.55
B	0.32	0.74
C	0.28	0.76
D	0.36	0.34
E	0.46	0.55
Average (A, C, E) of three	0.52	0.72
Average of five	0.40	0.75

indicating differences between Rieslings were appearance, color, aroma and bouquet, volatile acid, total acid, sugar, taste, general quality, and total. Many characteristics also discriminated between tasters. These characteristics were appearance, color, aroma and bouquet, volatile acid, total acid, sugar, body, taste, and tannin. The 2 kinds of wine produced quite different organoleptic responses. Color, acid, and sugar were much more important in distinguishing between Riesling wines than between Cabernets.

Scores obtained from estimating equations for these data range from *poor* to *moderately good*. With more extensive data the situation probably could be improved, at least for some tasters and products. There are many reasons why a better correspondence between observed and estimated scores was not obtained. They are discussed under the 3 headings—tasters, score cards, and estimating equations—that follow.

Tasters. From the taster's point of view there was the problem of being unable to taste all the samples at one time and to make comparisons between them. Furthermore, the tasters appear to have varied in skill. The coefficients of the linear estimating equations vary tremendously with taster and beverage.

Score cards. The score cards may not be revealing uniform differences between scores. Wood *(7)* has pointed out that the sum of the scores may be less desirable than the geometrical mean or some other function of the individual scores. We have no assurance, of course, that the score card gives due weight to each of the quality factors. In 1951 Amerine and Joslyn gave a series of score cards that have been proposed for wine—all differing slightly from each other. Boggs and Hanson *(3)* have criticized total scores but in judging over-all quality in wines it is difficult to avoid utilizing the total score. Another objection to the score card was that the tasters did not have uniform opinions regarding the scores for each part of the score card. On the color of Cabernet, for example, the judges marked very different scores for the same wine.

Estimating equations. Only a limited number of physical and chemical analyses were performed and only a few of these were used in the estimating equations. Quality in wines is probably a very complicated synthesis of many physical and chemical characters. The Cabernet and Riesling aromas are undoubtedly of great importance in the taster's mind in judging quality.

The remarkable fact is that with such a limited number of analyses and with so many faults made by the tasters and with the errors inherent in the score cards that such good correlations were sometimes obtained. Several correlations are statistically highly significant, but it is not possible to say just what the population values were because of the small number of wines available for each taster. The multiple correlation coefficients squared between the scores predicted by the estimating equations of those actually obtained for the 5 tasters on the Cabernets were 0.643, 0.322, 0.280, 0.362, and 0.641. For the Rieslings they were 0.546, 0.737, 0.761, 0.336, and 0.554. Equations for the Rieslings obviously are better than those for the Cabernet. One possible reason for this is that Cabernet aroma is a very important factor in its quality, but this character cannot at present be incorporated in the equations.

The results reported here for wines are poor as compared to results reported for tasting butter, for instance, where a standard score card has been in use for many years and the judges are highly trained. However, it is felt that a start has been made and that further study of more extensive data may lead to great improvement in the grading and marketing of beverages and possibly of other products.

LITERATURE CITED

1. AMERINE, M. A. (a) Organoleptic examination of wine, Proc. Wine Tech. Conf., Davis, Calif., pp. 15-26 (1948). (b) Laboratory procedures for enology, Univ. of California, Davis, 97 pp. (mimeo.) (1952).
2. BLANCHARD, E. L., AND MAXWELL, M. L. Correlation of subjective scoring with sugar content of frozen peas. *Food Research*, **6**, 105-115 (1941).
3. BOGGS, M. M., AND HANSON, H. L. Analysis of foods by sensory difference tests. *Advances in Food Research*, **2**, 219-258 (1949).
4. KLENK, E. Die Weinbeurteilung nach Farbe, Klarheit, Geruch und Geschmack des Weines. 1950, Eugen Ulmer, Stuttgart, 118 pp.
5. PALIERI, G., AND GAROGLIO, P. G. Il valori commerciale del vino e l'analisi organolettica. *Alimenti prod. agrar. e sost. agrar.*, **3**, 207-9 (1947).
6. WAGNER, K. G. Uber einige Zusammenhange zwischen organoleptischen und analytischen Befund bei Nahrungs mitteln. *Zeit. Lebensm.-Unters. u.-Forschung*, **89**, 241-50 (1949).
7. WOOD, E. C. Organoleptic tests in the food industry. III. Some statistical considerations in organoleptic tests. *Jour. Soc. Chem. Ind.*, **68**, 128-131 (1949).

ACETALDEHYDE AND RELATED COMPOUNDS IN FROZEN GREEN PEAS

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The relation of glycolytic changes *(20)*, leading to the accumulation of acetaldehyde and alcohol, to enzyme activity, and off-flavor formation, in unscalded and underscalded frozen vegetable tissue has been pointed out by Joslyn *(12)*.

The separation and identification of acetaldehyde, ethanol, and other volatile carbonyl compounds present in frozen peas and the conditions governing their formation were investigated in some detail by David *(7)* during the period, 1945-1949. The data on the accumulation of aldehydes and alcohol has been reported elsewhere *(13)*. The chromatographic separation of 2,4-dinitrophenylhydrazones of carbonyl compounds occurring in fresh and frozen pea seeds, their characterization by absorption spectroscopy, and related observations are reported here.

MATERIALS AND METHODS

Preparation of material. Freshly harvested Laxton Progress peas which had been frozen and stored since 1937 at —18°C., and various lots of fresh green peas bought locally were used. They were shelled by hand, mixed, washed, scalded in water for 2 minutes or more at various temperatures, cooled and frozen and stored in sealed cans.

Anaerobiosis was usually carried out at room temperature on approximately 400 g. of peas held in a stoppered 500 ml. Erlenmeyer flask under nitrogen for 6 hours. When aldehydes and alcohol alone were to be determined, the peas were surface sterilized by placing them in a 1% solution of mercuric chloride for 10-15 minutes after which they were washed twice with boiled distilled water. When carboxylase activity was to be determined, sterilization was not carried out. No bacterial contamination occurred under these conditions.

The volatile aldehydes and alcohol were obtained by steam distillation of 100 g. of finely ground material, until 400 ml. distillate was obtained.

Pyruvic carboxylase activity. Carboxylase activity was determined on a suspension prepared by blending 100 grams of fresh, partially blanched or frozen peas with 100 ml. of 0.125 M acetate buffer of pH 4.6 for 2½ minutes in a Waring blendor *(25)*. The ground material was then strained through 2 layers of cheese cloth and its pyruvic carboxylase activity was determined immediately, at 29.7°C. and pH 5.06-5.07, using the usual Warburg manometric technique.

Formation and separation of 2,4-dinitrophenylhydrazones (DNPH).[b] The acetaldehyde and other carbonyl compounds were separated from pea tissue by steam distillation, converted to the DNPHs, chromatographically separated first on magnesium sulfate, then on powdered silicic acid, and identified by absorption spectroscopy as was done for apricot extracts *(18, 27)*.

Identification and quantitative determination of acetyl methyl carbinol and diacetyl.[c] The presence of acetyl carbinol (A.M.C.) and diacetyl was tested by a modification of

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[b] Hereafter in this paper 2,4-dinitrophenylhydrazone will be abbreviated DNPH.

[c] Acetyl methyl carbinol or acetoin is abbreviated to AMC and diacetyl to DA in the text subsequently.

the Voges-Proskauer reaction *(23, 24)*. Acetyl methyl carbinol (A.M.C.) and diacetyl were determined quantitatively by a modification of the colorimetric method of White, *et al. (26)*, on an aliquot of steam distillate. Because of the presence of interfering substances, the clear filtrate resulting after addition of trichloroacetic or metaphosphoric acid could not be used. A.M.C. was oxidized before its determination by the procedure of Stotz and Raborg *(19)*. After cooling, 10 ml. of the oxidized mixtures were distilled and the diacetyl thus obtained was determined as above. When mixtures of A.M.C. and diacetyl were analyzed, A.M.C. was calculated as the difference between the oxidized and unoxidized samples.

Separation and identification of ethanol. Interfering carbonyl compounds present in the steam distillate were removed *(8, 9)* and the alcohol present was identified by the iodoform reaction and by the determination of the Duclaux constants of the acid obtained by oxidation with $K_2Cr_2O_7$ and concentrated H_2SO_4 *(17, 22)*.

RESULTS

Occurrence of acetaldehyde in frozen peas. The presence of acetaldehyde in fresh peas, as well as in peas submitted to anaerobiosis was shown by preparing the DNPH of acetaldehyde present in the distillate. The recrystallized DNPH from both preparations melted at a constant temperature of 167°C.[4] The same melting point was found for a preparation of the DNPH of pure acetaldehyde and for mixtures of the known with the unknown.

Supplementary evidence was supplied by a comparison of the absorption spectra of the unknown compound with that of the known derivative. Figure 1 shows that they are identical. A plot of the main band obtained on chromatography of the DNPH derivatives from fresh peas and of that obtained by the same procedure from peas submitted to anaerobiosis also gave curves similar to that of the pure acetaldehyde derivatives as can be seen from Figure 1. A mixed chromatograph of the known and unknown showed them to possess the same affinity for silicic acid since a separation of bands could not be observed.

On chromatographing the DNPHs derived from the carbonyl compounds present in frozen peas, one major component was always observed. Its absorption spectra corresponded closely to that of the acetaldehyde derivative except for slight differences observed occasionally which were probably due to the presence of non-volatile impurities in the eluting solvents. Mixed chromatographs did not show separation. These 2 observations were taken as evidence for the presence of acetaldehyde among the carbonyl compounds found in frozen peas.

Pyruvic carboxylase in peas. The pH optimum of pyruvic carboxylase in plant tissue has been reported to vary from 5.0 to 6.8 *(11, 21, 25)*. The data shown in Figure 2 indicate that for fresh pea tissue it is close to pH 5.15. It was found that a variation of as little as 0.14 of a pH unit produced about a 15% change in net evolution of carbon dioxide.

Shelled peas retain their carboxylase activity for a fairly long period of time when stored at 0°C. The CO_2 production, in μl per g. dry weight of pea seeds in 100 minutes was 1219.4, 1210.3 and 1105.9, respectively, immediately after shelling, after storage for 7 days and after storage for 10 days.

Freezing storage for a short period of time does not affect carboxylase activity. Raw peas stored at −18°C. for 15 days still showed about the same rate of carbon dioxide production as fresh peas. When frozen peas are placed at 0°C., several days after freezing, however, their ability to decarboxylate pyruvate disappears rapidly. Eleven days at 0°C. were sufficient to reduce the carbon dioxide production from 606.5 μl CO_2 per g. dry weight in 30 minutes for fresh peas to 143.7 μl for frozen peas stored at 0°C. The carboxylase activity of the raw peas after 11 years of freezing storage at −18°C., was about half of that of freshly frozen peas. The partially blanched samples did not show carboxylase activity after this long storage period.

Peas blanched at 54.5°C. for 2 minutes produced only 222.4 μl CO_2 per g. dry weight in 30 minutes as compared with 853.5 μl for the raw peas, while those blanched at 60°C.

[4] The values reported in the literature on the melting point of acetaldehyde DNPH vary from 147-169 because of isomerism *(1, 2, 4, 5, 6)*.

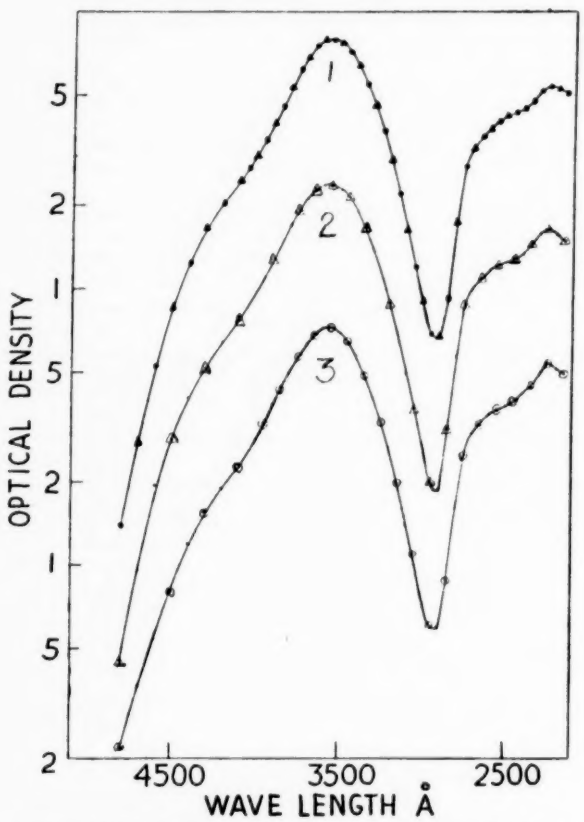

Figure 1. Absorption spectra of dinitrophenyl of pure acetaldehyde (curve 1) and of the acetaldehyde present in fresh pea seeds (curve 2) and of the acetaldehyde in peas held under anaerobic conditions. Solvent 95% ethanol.

produced only 30 μl in that same period of time, and those blanched at 71.1°C. did not produce any. When the peas of this series were allowed to respire in the absence of oxygen, the rate of carbon dioxide production increased for the raw peas and those blanched at 49° and 54.5°C. A decrease was observed for the 60°C. blanch.

An accumulation of aldehydes always occurred when partially blanched peas whose carboxylase activity was not completely destroyed were subjected to anaerobiosis. This increase in aldehyde content apparently is due to the production of acetaldehyde through the action of pyruvic carboxylase. The slight increase found for the peas blanched at 71.1°C. could also be due to the action of carboxylase present in a quantity not detectable by the Warburg technique.

Acetyl methyl carbinol and diacetyl. The bis-DNPH's of A. M. C. and D. A. were prepared, recrystallized from nitrobenzene and washed with alcohol and dried at 100°C. Their melting points were determined and found to be identical, which was expected, since from their structural formulas, an identical bis-DNPH should be obtained. They melted at about 315°C. after turning black around 300°C. This is similar to the reported melting point of the diacetyl bis-DNPH *(14)*. Both compounds were practically insoluble in ethyl alcohol. They gave a blue violet color in alkaline alcoholic solution. Their

absorption spectra in chloroform were identical, as can be seen from Figure 3 (curves 1 and 2). A comparison of the spectra of the known derivatives with that of the unknown showed only minor discrepancies in the shorter wave lengths and therefore provided strong evidence in favor of the identity of the two compounds. This was further substantiated by the finding that a mixed chromatograph did not show separation into 2 bands.

The steam distillates from fresh peas and peas submitted to anaerobiosis when tested qualitatively were positive for acetyl methyl carbinol and diacetyl. Assuming that little oxidation of the A.M.C. occurs during the steam distillation, it can be stated that both A.M.C. and D.A. are naturally present in fresh peas. Experimental results on peas respiring in the absence of oxygen lead to the same conclusion.

During the preparation of the bis-DNPH derivatives of A.M.C. and D.A. it was observed that the diacetyl derivative precipitated out readily while that of A.M.C. took a longer time to form. In this last case, the solution always turned to a deep orange color before any cloudiness appeared. The purified derivatives, however, were identical as already mentioned. In order to obtain more complete data on these carbonyl com-

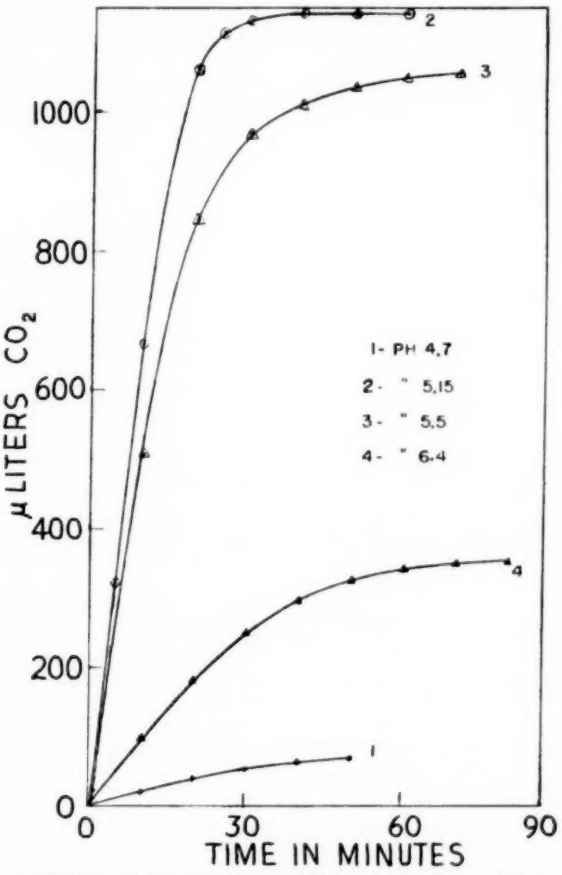

Figure 2. Effect of pH on carboxylase activity of peas. Microliters of CO_2 evolved per gram dry weight of pea tissue in 0.125 M acetate buffer.

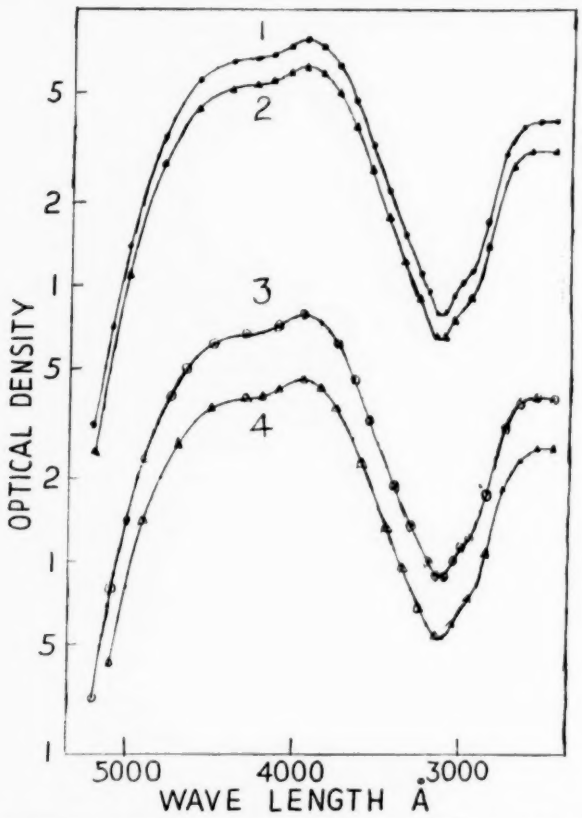

Figure 3. Absorption spectra of the bis-2-4 dinitrophenylhydrazones of acetyl methyl carbinol and diacetyl.

(1) Pure bis hydrazone of diacetyl; (2) pure bis hydrazone of acetyl methyl carbinol; (3) bis hydrazone obtained from fresh peas; (4) bis hydrazone obtained from fresh peas after anaerobiosis for 6 hours. Solvent: chloroform.

pounds, attempts were made to prepare their mono-DNPHs by adding the 2,4-dinitrophenylhydrazine in excess of the amounts required to form mono-derivatives from A.M.C. or D.A. A bright yellow precipitate could always be obtained readily with diacetyl. The A.M.C. preparations behaved very differently. As was the case for the bis-DNPH formation, the reaction mixture turned to an orange color on mixing the reagents and an orange precipitate came down slowly on standing. The diacetyl derivative could be recrystallized from an alcohol water mixture, melted at 175°C., and exhibited a deep red color in alkaline alcoholic solution. Its absorption spectrum, shown in Figure 4, was typical of a mono-DNPH. In comparison, the A.M.C. derivative was almost insoluble in alcohol and had to be recrystallized from nitrobenzene. It gave a blue violet color in alcoholic solution. Its identity with the bis-DNPH of A.M.C. was established by melting point determinations and spectral analysis.

Carboligase activity in peas. As can be seen from Table 1, peas possess a system capable of forming C_4 carbon chains from both pyruvate and acetaldehyde but pyruvate is a better substrate for this synthesis. Our results in which differential analyses were

TABLE 1

A. M. C. and diacetyl formation from added substrate by a pea preparation *

	Diacetyl + A.M.C	Diacetyl	A.M.C.
Peas bought locally November 5, 1948	μg	μg	μg
1. Fresh peas, no substrate	455	425	30
2. Fresh peas + 200 mg. pyruvate	1,895	1,895	20
3. Fresh peas + 200 mg. acetaldehyde	890	525	320
4. Fresh peas + 100 mg. pyruvate + 100 mg. acetaldehyde	882	775	107

*Formation of D.A. and A.M.C. in 100 gm. pea slurry containing 0.1% merthiolate as preservative incubated for 16 hours with pyruvate, acetaldehyde, or mixture of the two.

made indicate that in all the experiments diacetyl was present in larger amounts and that it was the only compound formed from added pyruvate. If acetaldehyde is used as substrate, the total production of the C_4 compounds is decreased to about half but a larger amount of A.M.C. is produced. Pyruvate apparently depresses the production of A.M.C. When both substrates are added together, the total weight being the same as

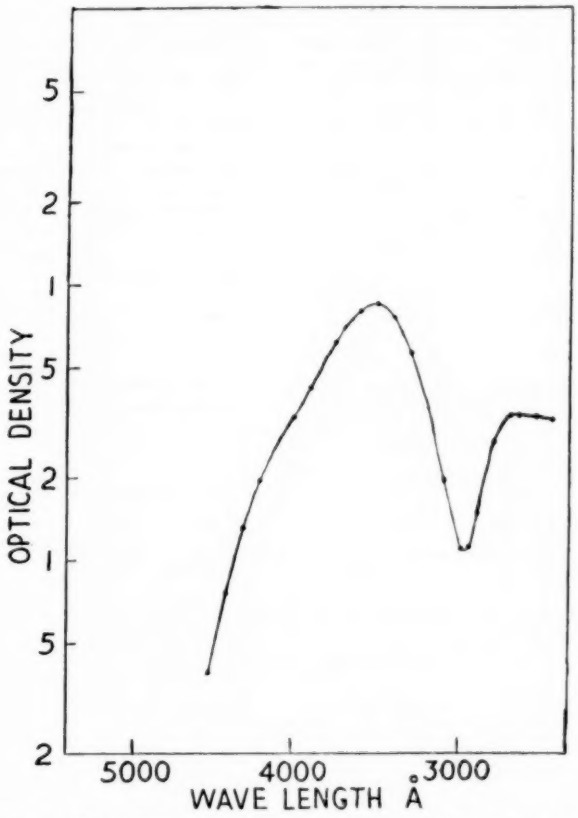

Figure 4. Absorption spectra of mono hydrazone of diacetyl in alcohol.

when they are used alone, the total amount of C_4 compounds does not vary greatly from that of acetaldehyde alone but the quantity of A.M.C. formed decreases considerably.

When pyruvate alone was used as substrate, only about 1% of it was converted to diacetyl. If A.M.C. originates mostly from acetaldehyde, one could expect a larger amount of it when pyruvate is added since acetaldehyde should be formed by decarboxylation. The pH of the slurry should be around 6.6 and it has been demonstrated in an earlier section that the rate of decarboxylation of added pyruvate is quite slow at pH 6.4. Although formed, acetaldehyde was probably not present in great enough concentration to favor A.M.C. formation. In addition, pyruvate has been found to exert an inhibitive action on the synthesis of A.M.C. from acetaldehyde when both substrates are added simultaneously. In this experiment pyruvate was always present in a much larger concentration than was acetaldehyde.

Occurrence of other carbonyl compounds. Several hydrazones were found to be present in both fresh peas and peas subjected to anaerobiosis. In order of their development on silicic acid after preliminary separation on magnesium sulfate, these were: (1) unknown 1; (2) acetaldehyde hydrazone; (3) unknown 2; (4) diacetyl hydrazone; (5) unknown 3; (6) unknown 4.

Unknown 1, obtained from fresh peas before and after anaerobiosis, and in peas scalded at 60°C. and 71.1°C. and stored for 8 years at −18°C., gave a red color in alkaline solution. It showed a maximum absorption at 3750Å and a minimum at 3000Å, indicating that the parent carbonyl compound is unsaturated and that it has a general formula of the type $X\,H\,N=C-C=C$ *(3, 16)*.

Unknowns 2 and 3 were present in larger amounts in peas subjected to anaerobiosis for 6 hours than in peas respiring in air. The diacetyl derivative appeared between these 2 unknowns on chromatography with silicic acid, using 80% benzene in petroleum ether as the eluant. The general appearance of the spectra of unknown 2 in peas before and after anaerobiosis suggests that they are identical compounds. Likewise, unknown 3 was apparently the same in both cases due to the close resemblance to both spectra and similar affinity for the adsorbent. The general characteristics of unknowns 2 and 3 resemble those of diacetyl bis-DNPH. Their ability to give a blue color in alkaline solution suggests α-dicarbonyl compounds or compounds capable of producing bis-DNPHs in which the two DNPHine molecules are in a 1,2 position.

The blue color in alkaline solution exhibited by unknown 4 and its broad, main band showing a peak of 4400Å again indicated a bis-DNPH of the α di-keto type.

Unknowns 2, 3 and 4 were easily soluble in alcohol which was used as the solvent for spectral analysis differing therefore in that respect from the diacetyl derivative.

TABLE 2

Identification of the acid obtained by oxidation of the alcohol present in fresh peas before and after anaerobiosis for 6 hours by the ordinary Duclaux method

Before anaerobiosis	After anaerobiosis for 6 hours	Duclaux no. for acetic acid
6.72	6.80	6.8
7.00	7.15	7.1
7.39	7.42	7.4

TABLE 3

Identification of the acid obtained by the oxidation of the alcohol present in fresh peas submitted to anaerobiosis for 6 hours and in unblanched frozen peas, by a modified Duclaux procedure

Fresh peas after anaerobiosis for 6 hours	Unblanched frozen peas stored at −18°C. 11 yrs.	Duclaux constants for acetic acid
15.4	15.7	15.7
32.4	32.8	32.7
51.1	51.1	51.4
72.2	72.8	72.6

Identification of alcohol in peas. The acid resulting from the oxidation of the steam distillate free from carbonyl compound was tested first by the ordinary Duclaux procedure and then by a modification of this procedure. Tables 2 and 3 give the results obtained by these 2 procedures. They show that ethyl alcohol is apparently the only alcohol present in peas.

SUMMARY

Acetaldehyde was identified in the steam distillate of fresh peas, fresh peas subjected to anaerobiosis, and frozen peas.

The presence of pyruvic carboxylase was demonstrated in peas. It was most active at pH 5.1 and and was not inhibited by freezing. Pea carboxylase was inactivated by heating peas at 60°C. for 2 minutes.

Acetyl methyl carbinol and diacetyl were identified in the steam distillate of fresh peas, fresh peas subjected to anaerobiosis, and frozen peas. In addition, at least 4 other carbonyl compounds were present, but these occurred in quantities too small to permit their identification.

Diacetyl was shown to accumulate in presence of added pyruvate and but little was formed in presence of added acetaldehyde, indicating that diacetyl was formed preferentially from pyruvate.

Acetyl methyl carbinol was formed largely from added acetaldehyde rather than added pyruvate, but in amounts considerably below those of diacetyl.

Ethyl alcohol was the only alcohol present in the steam distillate of peas.

LITERATURE CITED

1. Allen, C. F. H. The identification of carbonyl compounds by use of 2,4-dinitrophenylhydrazine. *J. Am. Chem. Soc.*, **52**, 2955 (1930).
2. Allen, C. F. H., and Richmond, J. H. Some limitations of 2,4-dinitrophenylhydrazine as a reagent for carbonyl compounds. *J. Org. Chem.*, **2**, 222 (1937).
3. Braude, E. A., and Jones, R. H. Studies in light absorption. II. 2,4-dinitrophenylhydrazones. *J. Chem. Soc.*, **1945**, 498 (1945).
4. Brady, O. L. The use of 2,4-dinitrophenylhydrazine as a reagent for carbonyl compounds. *J. Chem. Soc.*, **1931**, 756 (1931); see also *Analyst*, **51**, 77 (1926).
5. Bryant, W. M. D. Optical crystallography of acetaldehyde 2,4-dinitrophenylhydrazone. *J. Am. Chem. Soc.*, **55**, 3201 (1933). See also *ibid.*, **58**, 2335 (1936); **60**, 2814 (1938).
6. Campbell, N. R. The use of 2,4-dinitrophenylhydrazine as a reagent for carbonyl compounds. *Analyst*, **61**, 391 (1936).
7. David, J. J. Acetaldehyde and related carbonyl compounds in peas. Ph.D. Thesis, Univ. Calif., 1949.
8. Friedman, T. E. The identification and quantitative determination of volatile alcohols and acids. *J. Biol. Chem.*, **123**, 161 (1938).
9. Friedman, T. E., and Klaas, R. The determination of ethyl alcohol. *J. Biol. Chem.*, **115**, 47 (1936).
10. Horowitz, N. H., and Heegaard, E. Experiments on the carboxylase of pea roots. *J. Biol. Chem.*, **137**, 475 (1941).
11. James, W. O., and Norval, I. P. The respiratory decomposition of pyruvic acid by barley. *New Phytol.*, **37**, 455 (1938).
12. Joslyn, M. A. Enzyme activity in frozen vegetable tissues. *Advances in Enzymology*, **9**, 631 (1949).
13. Joslyn, M. A., and David, J. J. Acetaldehyde and alcohol in raw or underblanched peas. *Quick Frozen Foods*, **15** (No. 4), 51 (1952).
14. Neuberg, C., and Strauss, E. Microdetermination of carboxyl compounds. On the coloring of nitro compounds in alkaline solutions. *Arch. Biochem.*, **7**, 457 (1945).

15. OCHOA, S. Biological mechanisms of carboxylation and decarboxylation. *Physiol. Rev.*, **31**, 56 (1951.)
16. ROBERTS, J. D., AND GREEN, C. Separation of 2,4-dinitrophenylhydrazones by chromatographic adsorption. *Ind. Eng. Chem., Anal. Ed.*, **18**, 335 (1946).
17. SHRINER, R. L., AND FUSON, R. C. *The Systematic Identification of Organic Compounds.* 1940, John Wiley and Sons, Inc., New York.
18. STADTMAN, F. H. The chemical deterioration of dried fruit during storage. III Chromatographic separation of carbonyl compounds as 2,4-dinitrophenylhydrazones. *J. Am. Chem. Soc.*, **70**, 3583 (1948).
19. STOTZ, E., AND RABORG, J. A colorimetric determination of acetoin and diacetyl. *J. Biol. Chem.*, **150**, 25 (1943).
20. STUMPF, P. K. Glycolytic enzymes in higher plants. *Ann. Rev. Plant Physiol.*, **3**, 17 (1952).
21. TANKO, B., AND MUNK, L. Beiträge zum Carboligaseproblem. *Z. Physiol. Chem.*, **262**, 144 (1939).
22. VAN DER LEK. Onderzvekinger over den butyl alkolgesting. Ph.D. Diss. Delft (1930).
23. VAUGHN, R. H., AND LEVINE, M. Differentiation of the intermediate coli-like bacteria. *J. Bact.*, **44**, 487 (1942).
24. VAUGHN, R. H., WEDDING, G. T., AND TABACHNICK, J. The Voges-Proskauer reaction and differentiation of the coliform bacteria. *Hilgardia*, **18**, 357 (1948).
25. VENNESLAND, B., AND FELSHER, R. Z. Oxalacetic and pyruvic carboxylase in some dicotyledonous plants. *Arch. Biochem.*, **11**, 279 (1946).
26. WHITE, A. G. C., KRAMPITZ, L. O., AND WERKMAN, C. H. Methods for the direct determination of diacetyl in tissue and bacterial filtrates. *Arch. Biochem.*, **9**, 229 (1945).
27. WAHHAB, A. Deterioration of dried fruits. II. Identification of furfurals. *J. Am. Chem. Soc.*, **70**, 3580 (1948).

VARIETAL SUSCEPTIBILITY OF WHITE WINES TO BROWNING. I. ULTRAVIOLET ABSORPTION OF WINES

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Undesirable changes in color and palatability often occur during the storage of white wines. These changes have been ascribed to oxidative storage deterioration *(1)*. Among the constituents that have been considered to be involved in this browning are the naturally occurring phenolic substances usually considered to be "tannins." Johnson, Foreman, and Mayer *(3)* have reported the occurrence of at least two or more types of polyphenolic substances extracted from various fruits. One type is characterized by an absorption maximum at 280 mμ, the other at 322-324 mμ. Williams and Wender *(5)* have reported the presence of quercetin and isoquercitrin in Tokay, Emperor, and Thompson Seedless grapes. To obtain some indication of the types of compounds that might be present in white wines and to determine the relation of "tannin" content to susceptibility to darkening a number of wines made from several varieties of white wine grapes at several stages of maturity were examined spectroscopically.

MATERIALS AND METHODS

Approximately 50-pound lots of grapes were crushed in a small standard paddle- and screen-type crusher equipped with stem separator and pressed immediately after crush-

TABLE 1

Optical density range of all wines in the 5 groups

Group	Variety	Samples	250 mμ	260-265 mμ	320-325 mμ	340 mμ
1	Aligote	4	0.183-0.227	0.178-0.196	0.097-0.201	0.072-0.153
	Emerald Riesling	4	0.150-0.161	0.149-0.155	0.051-0.100	0.045-0.083
	White Riesling	4	0.188-0.229	0.182-0.218	0.148-0.210	0.112-0.160
2	Pinot blanc	3	0.203-0.282	0.177-0.240	0.206-0.317	0.161-0.238
3	Palomino	3	0.130-0.189	0.127-0.191	0.079-0.119	0.058-0.091
	Red Veltliner	3	0.135-0.193	0.138-0.193	0.076-0.101	0.062-0.096
4	Muscat Canelli	3	0.159-0.172	0.153-0.169	0.120-0.139	0.092-0.105
	Sauvignon blanc	3	0.164-0.212	0.169-0.212	0.092-0.110	0.073-0.089
5	Burger	2	0.114-0.135	0.107-0.124	0.086-0.089	0.063-0.072
	Chardonnay	3	0.179-0.213	0.178-0.209	0.095-0.123	0.079-0.098
	Chenin blanc	3	0.136-0.154	0.136-0.161	0.072-0.096	0.058-0.074
	Folle blanche	2	0.124-0.131	0.126-0.144	0.063-0.073	0.046-0.054
	French Colombard	3	0.174-0.180	0.166-0.183	0.066-0.096	0.060-0.078
	Gewurz Traminer	3	0.145-0.169	0.153-0.186	0.079-0.094	0.061-0.077
	Sauvignon vert	3	0.118-0.159	0.117-0.167	0.065-0.068	0.050-0.053
	Semillon	3	0.164-0.189	0.161-0.184	0.076-0.091	0.066-0.075
	Sylvaner	4	0.143-0.186	0.145-0.195	0.087-0.110	0.064-0.083
	Thompson Seedless	3	0.116-0.136	0.126-0.136	0.054-0.062	0.048-0.049
	Tokay	4	0.119-0.137	0.120-0.133	0.052-0.064	0.039-0.054

ing. The juice was collected in glass containers, 100 p.p.m. of sulfur dioxide added, and the juice allowed to stand until the following morning when it was racked from the gross lees. A 1% culture of Montrachet strain yeast was added and the juice allowed to ferment to dryness at approximately 14.4°C. The wine was then racked into bottles, corked, and allowed to stand at 12.8°C. for one month when it was again racked. Two months after completion of the fermentation each lot was filtered through diatomaceous earth and then analyzed.

All of the wines were diluted 50 times with water for the ultra-violet absorption determinations on the Beckman Model DU quartz spectrophotometer, using a hydrogen discharge lamp as the light source and 10 mm. quartz cuvettes. The Loewenthal titration *(4)* was used in the tannin determination.

RESULTS AND DISCUSSION

The UV absorption spectra of the wines made from the 19 varieties of *Vitis vinifera* white wine grapes fell into one of 5 type groups, shown in Figures 1-5 and summarized in Table 1. In general absorption maximums occurred in the regions of 260-265 and 315-325 mμ. While the curves are in

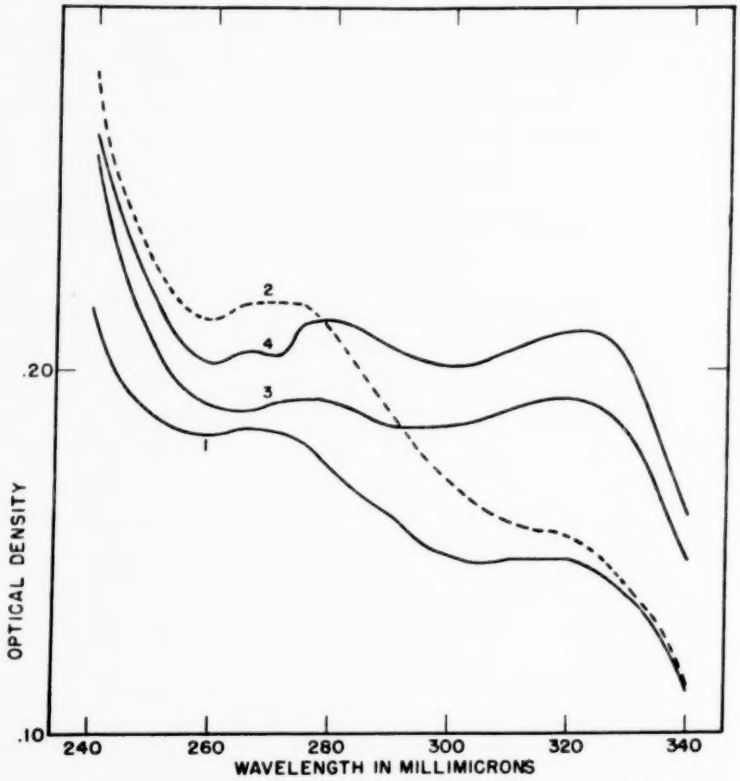

Figure 1. Absorption spectra of group 1 wines. 1. High maturity White Riesling. 2. High maturity White Riesling. 3. Low maturity White Riesling and 4. Low maturity White Riesling.

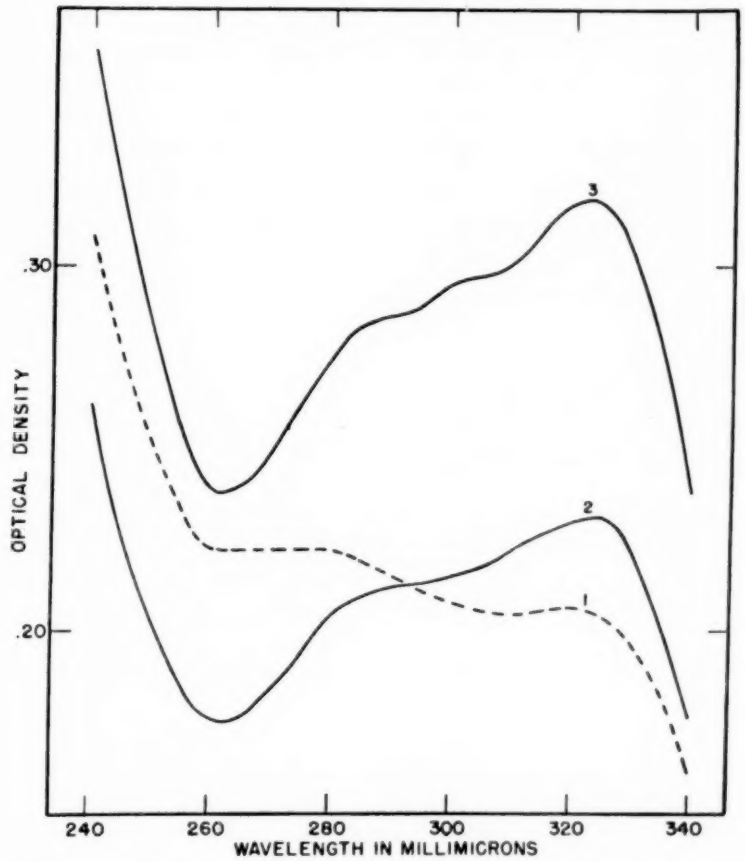

Figure 2. Absorption spectra of group 2 wines. 1. Intermediate maturity Pinot blanc. 2. Low maturity Pinot blanc and 3. High maturity Pinot blanc.

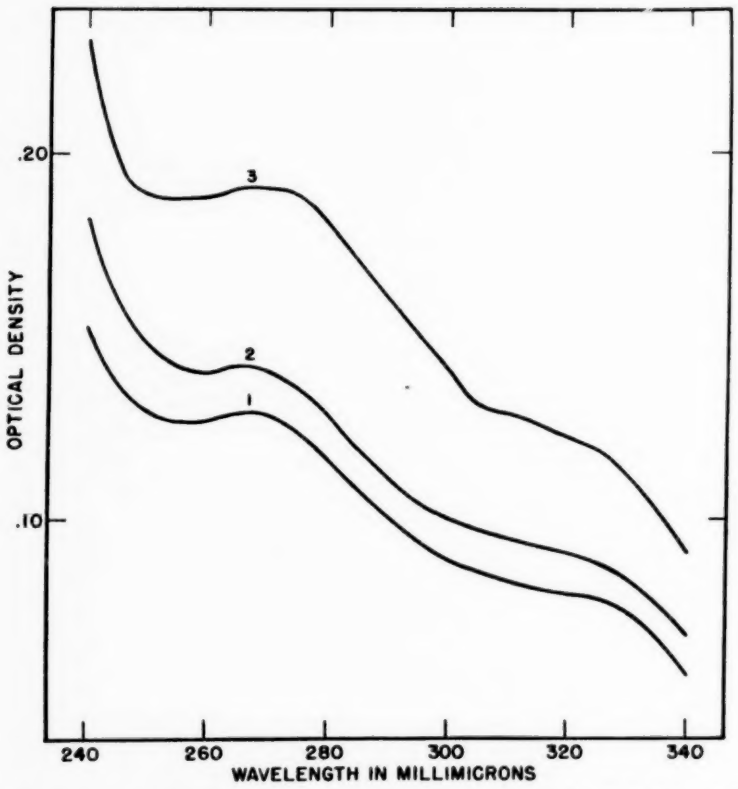

Figure 3. Absorption spectra of group 3 wines. 1. Low maturity Palomino. 2. Intermediate maturity Palomino and 3. High maturity Palomino.

general similar to those obtained by Johnson, Foreman, and Mayer *(3)* they represent the absorption of possible phenolic substances modified by the presence of other constituents of the wine. Wines in group 1 definitely show effect of maturity. The wines from grapes harvested early at a lower °Balling have a minima at 260-265, a maxima at 275-280 and a marked maxima at 320-321 mμ, while the wines made from grapes harvested later at a higher °Balling have a maxima at 265-270 and slight or no maxima at 320 mμ. Group 2 wines also show a maturity effect with those of lowest and highest maturity having a sharp minima at 261-262 and an equally sharp maxima at 323-324 mμ with the wine of intermediate maturity having a slight maxima at 260-280 and at 320 mμ. Group 3 wines have a maxima at 265-267 mμ only. Group 4 wines have a maxima at 255 or 263-265 and at 315-320 mμ. Group 5 wines have maxima at 265 and may or may not have maxima at 320 mμ. It is possible that catechins, anthocyanins, and chlorogenic acid constituents occur in white wine, but investigation of the types of phenolic substances present was not made at this time.

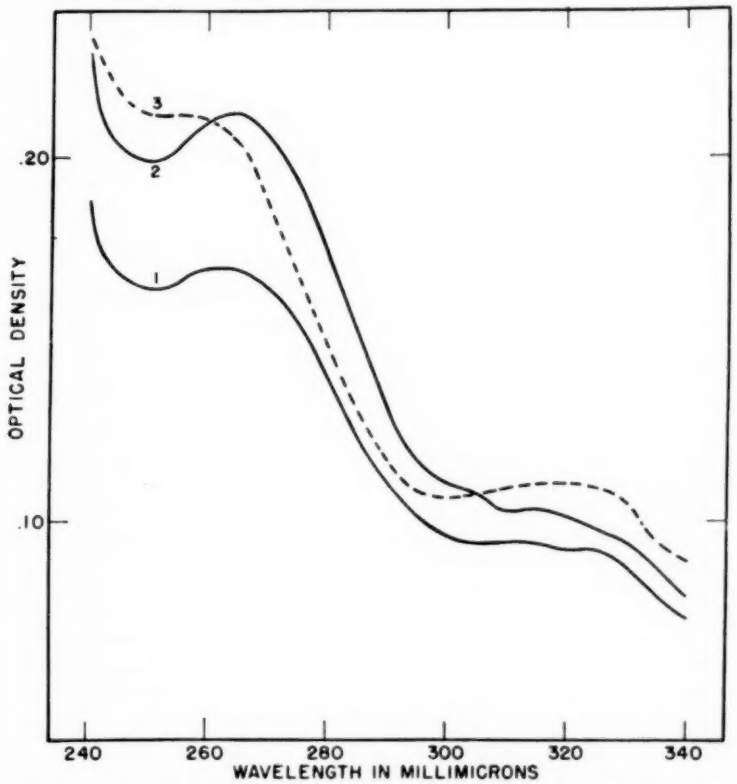

Figure 4. Absorption spectra of group 4 wines. 1. High maturity Sauvignon blanc. 2. High maturity Sauvignon blanc and 3. Low maturity Sauvignon blanc.

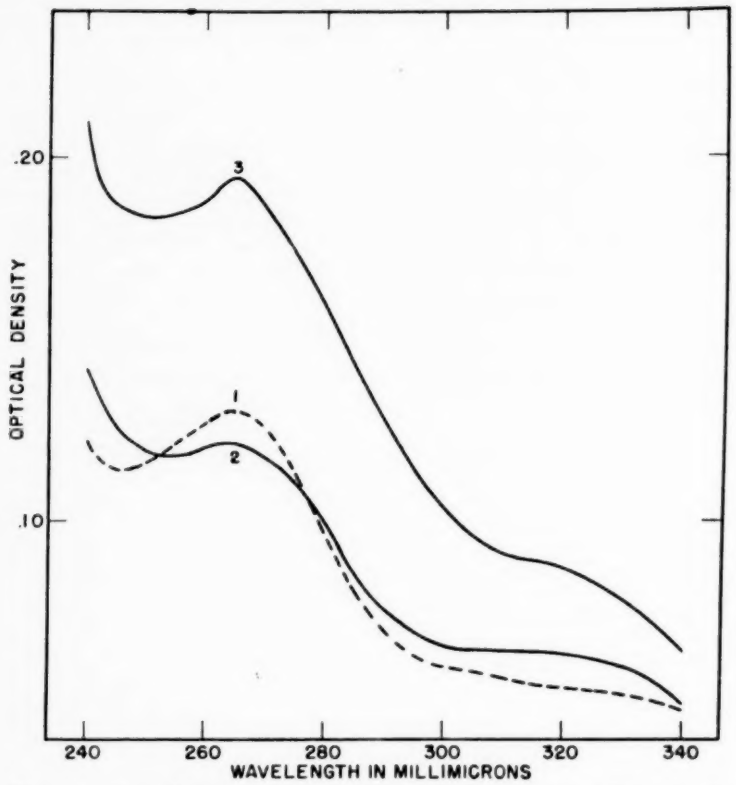

Figure 5. Absorption spectra of group 5 wines. 1. Low maturity Thompson Seedless. 2. Low maturity Tokay and 3. High maturity Sylvaner.

A relationship was found between the tannin content of the wine and the optical density at three wavelengths. The coefficient of correlation was +0.84 with the optical density at 265 mμ, +0.79 at 280 mμ and +0.63 at 320 mμ. The relationship at 265 mμ is shown in Figure 6. That the best correlation was obtained with the optical density at 265 mμ instead of 280 as found by Johnson, Foreman, and Mayer *(3)* was probably due to differences in varietal behavior, differences in method of tannin analysis, or to a shift caused by previous lead precipitation of the phenolic substances.

Heating the wines under specified conditions *(2)* affects the UV absorption and the color. The absorption as shown in Figure 7 is increased at 240-297 mμ and may be increased, decreased, or remain approximately the same at 298-340 mμ. The amount of the change in absorption varies considerably, with the greatest changes usually occurring in the regions of 265 and 320 mμ. All the wines when heated darken, with the degree of darkening varying greatly. In general the UV absorption of the unheated wines is closely related to the degree of darkening exhibited by the heated wines.

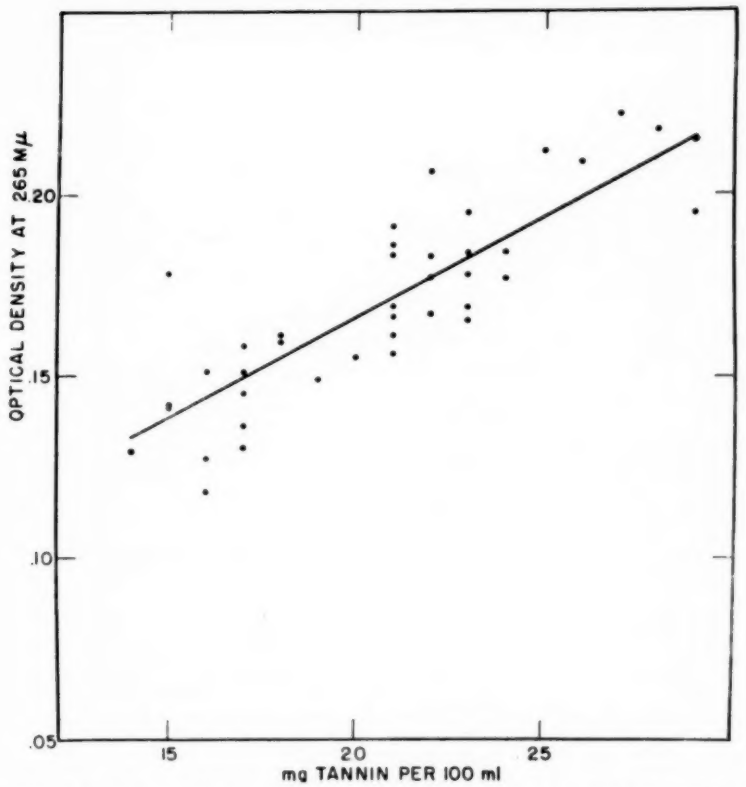

Figure 6. The relationship between UV absorption at 265 mμ and the amount of tannin as determined by the Loewenthal titration.

If the wines are considered within their respective groups usually the greater the optical density at 265 mμ the greater the browning on heating. Marked absorption in the region of 265 mμ enhances the browning effect as does minimum absorption at 320-325 mμ, while wines showing a marked absorption at 320-325 usually are resistant to darkening even when the optical density at 265 mμ is high or the absorption at that wavelength is marked. Without exception all the wines having an optical density of 0.14 or less at 265 mμ were resistant to darkening. The resistance to darkening of 83% of the wines exceeding 0.14 was directly dependent upon the relative absorptions at 260-265 and 315-325 mμ.

SUMMARY

The wines made from 19 varieties of *Vitis vinifera* white grapes were found to give 5 types of UV absorption curves which showed either maxima or minima in the region of 260-280 mμ with often another maxima in the region of 315-325 mμ. A significant relationship was found between the

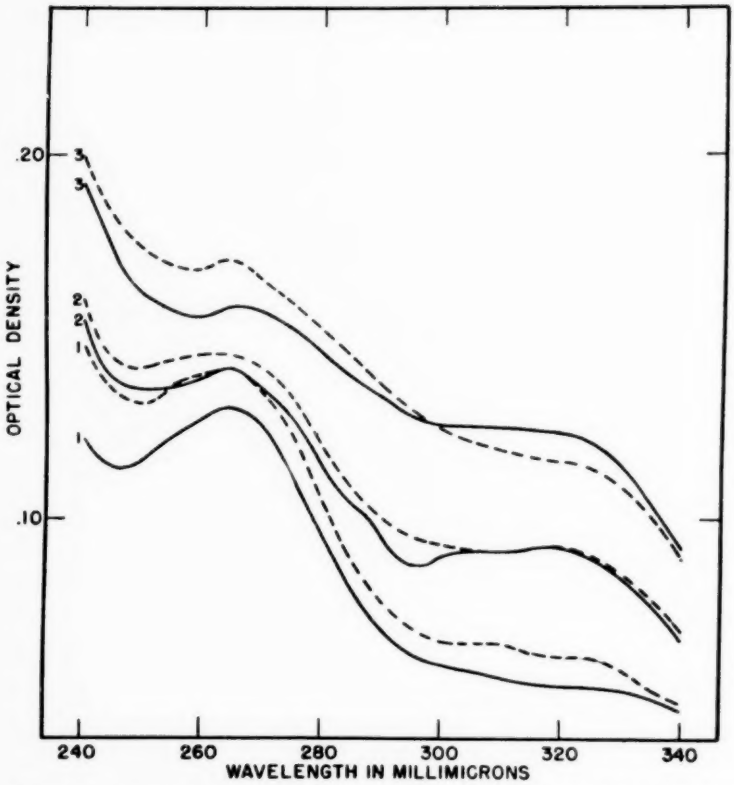

Figure 7. Absorption spectra of heated (broken lines) and unheated (solid lines) wines. 1. Thompson Seedless. 2. Red Veltliner and 3. Aligote.

amount of phenolic substances as determined by the Loewenthal titration and the UV absorption at 265 mμ of these substances. Heating the wines caused an increase in the UV absorption in the region 240-297 mμ and an increase, decrease or practically no change in the region 298-340 mμ. A relationship was found between the relative UV absorption at 260-265 and 315-325 mμ and the susceptibility of the wines to browning.

LITERATURE CITED

1. AMERINE, M. A., AND JOSLYN, M. A. *Table Wines.* 1951, University of California Press, Berkeley, California.
2. BERG, H. W. Varietal susceptibility of white wines to browning. II. Accelerated storage tests. *Food Research,* **18,** (4) 407 (1953).
3. JOHNSON, GESTUR, FOREMAN, E. M., AND MAYER, M. M. The ultraviolet absorption of polyphenolic substances from various fruits. *Food Technol.,* **4,** 241 (1951).
4. JOSLYN, M. A. *Food Analysis.* 1950, Academic Press Inc., New York, N. Y.
5. WILLIAMS, BYRON L., AND WENDER, SIMON, H. The isolation and identification of quercetin and isoquercitrin from grapes *(Vitis vinifera). J. Am. Chem. Soc.,* **74,** 4372 (1952).

VARIETAL SUSCEPTIBILITY OF WHITE WINES TO BROWNING. II. ACCELERATED STORAGE TESTS

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Although the relative tendency of wines made from certain varieties of grapes to oxidative browning has been observed industrially *(2)* few if any controlled experiments designed to determine this have been made *(4)*. In order to determine whether or not a correlation could be established between the grape variety and the darkening in color of the resultant wine, a number of wines from several varieties were made during the 1950 and 1951 vintages and subjected to an accelerated storage test.

MATERIALS AND METHODS

The wines were made as described previously, Berg *(3)*. A total of 106 wines were made from 19 varieties of white grapes. These included most of the varieties commonly used in the production of white table wines in California. In 1950 wines were made only from grapes grown at Davis, whereas in 1951 grapes grown at both Davis and in the Napa Valley were used. Grapes were selected from the two localities in 1951 to determine if region exerted an influence on varietal behavior as evidenced by change of color of the resultant wines. The principal climatic difference between the 2 regions is the average temperature during the growing season—Davis having an average of 3600 and Napa 2400 degree days of temperature (summation of average degrees above 50°F. for each day during the growing season). From one to 4 lots of wine were made from each variety in both 1950 and 1951.

The optical density of the filtered wines at a wavelength of 425 mμ was determined using a 20 ml. deep cuvette in a Coleman Model No. 11 spectrophotometer. Two hundred ml. of each wine was pipetted into standard fifth gallon (775 ml.) wine bottles which were tightly corked and stored at 48.9°C. for 7 days. The headspace provided approximately 600 ml. of oxygen per liter of wine, greatly in excess of that which the wine would absorb and fix *(5)*. At the end of this heating period the wines were removed from the hot room, cooled to room temperature, filtered when necessary and the optical density at 425 mμ again determined.

That the browning incurred by the wines under the conditions of the accelerated storage test is related to the rate of browning occurring under usual storage conditions is partly confirmed by 2 experiments designed to determine the effect of various temperatures and amounts of available oxygen on the browning rate. In one it was found that with 3 varietal wines, sealed in bottles containing 600 ml. atmospheric oxygen per liter of wine, the rate of darkening at 48.9°C. was respectively 17.8, 21.0, and 21.7 times that of the same wines with 7 ml. available oxygen per liter stored at 12.8°C. In the second experiment the rate of darkening was found to be proportional to the headspace. With 600, 12 and 7 ml. available oxygen per liter of wine the time required at 48.9°C. for the optical density to increase 0.150 was respectively 2.8, 18.0, and 25 days.

The upper limit of commercial acceptability for white table wines was found by examination to be at an optical density of 0.330 at 425 mμ. This limit for darkening is determined by initial pigment content of the wine and the increase in pigmentation on aging.

The pH, total acidity, tannin content and Balling degree were determined by methods given in the section on wines in the A.O.A.C. *(1)*.

RESULTS AND DISCUSSION

The average optical density of all lots of wines of each variety before and after heating is shown in Table I where the varieties are arranged in

TABLE 1

Varietal order of susceptibility to browning

Variety	No. of Samples	Optical Density Before Heating				Optical Density After Heating				% Excessively Dark[b]
		Max.	Min.	Ave.	S.D.[a]	Max.	Min.	Ave.	S.D.[a]	
Pinot blanc	5	0.427	0.218	0.292	0.085	0.653	0.337	**0.460**	0.136	100.0
Palomino	6	0.365	0.168	0.236	0.073	0.541	0.272	0.400	0.097	83.3
Emerald Riesling	5	0.321	0.188	**0.253**	**0.064**	0.787	0.280	0.438	0.186	80.0
Sylvaner	8	0.322	0.152	0.220	0.056	0.563	0.217	**0.363**	**0.116**	62.5
Chardonnay	7	0.336	0.181	0.227	0.061	0.600	0.247	0.360	0.113	57.1
Semillon	7	0.219	0.117	0.186	0.037	0.523	0.194	0.352	0.114	57.1
White Riesling	9	0.375	0.161	**0.237**	**0.059**	0.563	0.251	**0.378**	**0.123**	55.6
Tokay	2	0.169	0.165	0.167	0.003	0.697	0.222	0.460	0.338	50.0
Folle blanche	2	0.393	0.207	0.300	0.131	0.398	0.285	0.342	0.080	50.0
Red Veltliner	4	0.252	0.158	0.196	0.041	0.457	0.229	0.328	0.096	50.0
Sauvignon blanc	7	0.259	0.148	0.179	0.037	0.456	0.190	**0.341**	**0.096**	42.9
Sauvignon vert	5	0.309	0.131	0.194	0.069	0.631	0.222	0.388	0.185	40.0
French Colombard	8	0.243	0.137	**0.174**	**0.043**	0.668	0.194	**0.329**	**0.148**	37.5
Aligote	6	0.289	0.150	0.196	0.051	0.477	0.207	0.305	0.109	33.3
Chenin blanc	6	0.261	0.117	0.180	0.052	0.538	0.214	0.303	0.124	33.3
Thompson Seedless	4	0.149	0.124	0.134	0.012	0.375	0.208	0.254	0.081	25.0
Muscat Canelli	6	0.265	0.154	**0.182**	**0.043**	0.365	0.204	0.263	0.056	16.7
Gewurz Traminer	7	0.242	0.159	0.193	0.029	0.371	0.222	0.280	0.053	14.3
Gray Riesling	2	0.211	0.197	0.204	0.010	0.330	0.320	0.325	0.007	0.0

[a] Standard deviation.
[b] Percent of samples exceeding 0.330 optical density after heating.

order of susceptibility to browning. As shown in column 11, Pinot blanc was most susceptible to browning and Grey Riesling least. There was considerable variation between samples, particularly noticeable in Tokay, Emerald Riesling and Sauvignon vert wines. It is interesting to note that in general the varieties considered superior for winemaking were less resistant to browning.

Table 2 shows the effect of region and season on the susceptibility of the

TABLE 2

Effect of region and season on varietal susceptibility to browning

Variety	Percent of samples exceeding 0.330 optical density after heating		
	Davis 1950	Davis 1951	Napa 1951
Pinot blanc	100.0 (1)	100.0 (2)	100.0 (2)
Palomino	100.0 (3)	50.0 (2)	100.0 (1)
Emerald Riesling	100.0 (2)	50.0 (2)	100.0 (1)
Sylvaner	75.0 (4)	100.0 (1)	33.3 (3)
Chardonnay	66.6 (3)	50.0 (2)	50.0 (2)
Semillon	100.0 (3)	50.0 (2)	0.0 (2)
White Riesling	75.0 (4)	66.6 (3)	0.0 (2)
Tokay	0.0 (1)	100.0 (1)	〃
Folle blanche	〃	100.0 (1)	0.0 (1)
Red Veltliner	100.0 (1)	100.0 (1)	0.0 (2)
Sauvignon blanc	66.6 (3)	0.0 (2)	50.0 (2)
Sauvignon vert	33.3 (3)	100.0 (1)	0.0 (1)
French Colombard	0.0 (2)	50.0 (4)	50.0 (2)
Aligote	50.0 (2)	50.0 (2)	0.0 (2)
Chenin blanc	0.0 (2)	50.0 (2)	50.0 (2)
Thompson Seedless	0.0 (2)	50.0 (2)	〃
Muscat Canelli	0.0 (2)	0.0 (2)	50.0 (2)
Gewurz Traminer	0.0 (3)	33.3 (3)	0.0 (1)
Gray Riesling	〃	〃	0.0 (2)

Note: Numbers in parentheses refer to numbers of wines in the group.

various varieties to browning. The response of the individual varieties is not consistent, though there is less tendency to browning of the wines from the Napa grapes.

Since variety of grape from which made has been shown to have a decided effect on the susceptibility of a wine to browning, it was felt that the pH, total acidity and tannin content of the must, which, in the same maturity range is largely a varietal characteristic, might be correlated directly with browning. To test this theory the pH, total acidity and tannin content of the musts of the 3 groups of wines were correlated with the optical densities of the resultant unheated wines and with the increase in optical density after heating (Table 3). With the unheated wine a correlation at

TABLE 3

Correlation of certain constituents of the must with the optical density of the unheated wines and with the increase in optical density on heating

Must Constituent	Maximum	Minimum	Average	Unheated		Increase on Heating	
				r[c]	P[d]	r[c]	P[d]
Davis Musts—1950[e]							
Total Acid	1.57	0.41	0.77	−0.317	0.05	−0.113	0.50
pH	3.82	3.06	3.48	0.339	0.05	0.432	0.01
Davis Musts—1951[f]							
Total Acid	1.41	0.41	0.81	−0.288	0.10	−0.203	0.20
pH	3.75	3.09	3.49	0.388	0.05	0.205	0.30
Tannin	0.047	0.004	0.025	0.265	0.20	−0.355	0.05
Napa Musts—1951[g]							
Total Acid	1.27	0.43	0.74	0.015	0.50	0.228	0.30
pH	3.68	2.86	3.29	0.049	0.50	−0.112	0.50
Tannin	0.056	0.010	0.027	0.736	0.01	0.633	0.01

[c] Coefficient of correlation.
[d] Percent probability level—obtained from Youden's *Statistical Methods for Chemists*, p. 119.
[e] 41 samples.
[f] 34 samples.
[g] 31 samples.

the 5% level, or better, of significance was obtained with total acidity and pH in the 1950 Davis series, with pH only in the 1951 Davis series, and with tannin only in the 1951 Napa series. With the heated wines a correlation at the 5%, or better, level of significance was obtained with pH in the 1950 Davis series, with tannin in the 1951 Davis series, and with tannin in the 1951 Napa series. However, it is noted that one series gives a positive correlation with tannin and the other a negative.

As fermentation usually results in significant changes in the pH, total acidity and tannin content it was felt that the pH, total acidity and tannin content of the wines should be correlated with browning. Table 4 gives the correlation of these factors with the increase in optical density of the heated wines. With the 1950 Davis wines a correlation at the 5%, or better, level of significance was obtained with the pH, in the 1951 Davis series with both the pH and total acidity, and in the 1951 Napa series with the tannin.

Consistent correlation of the pH, total acidity or tannin content of either the must or wine with the optical density of the wines was not ob-

TABLE 4

Correlation of certain constituents of the wine with the increase in optical density of the heated wines

Wine Constituent	Maximum	Minimum	Average	r[c]	P[d]
Davis Wines—1950[a]					
Total Acid	1.31	0.38	0.69	−0.151	0.40
pH	4.15	2.96	3.51	0.464	0.01
Tannin	0.029	0.014	0.021	0.213	0.20
Davis Wines—1951[b]					
Total Acid	1.16	0.36	0.66	−0.470	0.01
pH	4.08	3.01	3.50	0.462	0.01
Tannin	0.042	0.011	0.020	−0.289	0.10
Napa Wines—1951[a]					
Total Acid	1.26	0.47	0.74	0.286	0.20
pH	3.65	2.72	3.20	−0.111	0.50
Tannin	0.051	0.001	0.017	0.607	0.01

tained. This is not surprising, however, in view of the fact that quite often 2 wines, each made from the same variety of grape from the same vineyard in the same year and of approximately the same gross chemical composition, will differ appreciably in their susceptibility to darkening. This difference is usually reflected in the UV absorption spectra, Berg *(3)*, of the two wines, though this does not always account for the variation. Thus we are forced to conclude that a number of factors, some known and some probably still unknown, are influencing browning. Some of these factors are without question directly related to variety, though probably modified by seasonal and regional effects.

SUMMARY

The variety of grape is related to the susceptibility of its wines to browning. Nineteen varieties of white wine grapes have been classified in the order of increasing resistance of their wines to browning. This order, which is based on a limited number of samples, is tentative and is subject to modification when additional data are obtained. Seasonal variations and region where grown have an effect on a given variety's susceptibility to browning. The pH, total acidity and tannin content of the must do not consistently correlate with susceptibility of the resultant wines to browning. The pH, total acidity and tannin content of the wines do not consistently correlate with their susceptibility to browning.

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 6th Ed. 1945, Association of Official Agricultural Chemists, Washington, D. C.
2. Berg, Harold W. The problem of oxidation of white wines. Wine Institute, San Francisco (mimeo).
3. Berg, H. W. Varietal susceptibility of white wines to browning. I. Ultraviolet absorption of wines. *Food Research*, **18**, (4) 399 (1953).
4. Ribéreau-Gayon, J. Contribution à l'étude des oxydations et réductions dans les vins. 2nd Ed., 1933. Delmas, Bordeaux, France.
5. Genevois, L., and Ribéreau-Gayon, J. *Le Vin*. 1947, Herman & Cie, Paris, France.

HEAT RESISTANCE OF CAMERON'S PUTREFACTIVE ANAEROBE 3679 IN PHOSPHATE BUFFER *(CLOSTRIDIUM SPOROGENES)*

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It is well known that the greater the concentration of spores in a medium, the greater their heat resistance; thus, either more heat or a longer process time is required to complete sterilization. The causes for this are not sufficiently explained. Rahn *(20)* described the fact from the standpoint of logarithmic order of death of bacterial spores by moist heat. Nevertheless, discrepancies from the logarithmic order of death are frequently observed. Bullei *(8)* found that the time required for killing *Staphylococcus aureus* increased with an increase in the number of cells, and he concluded that the time relationships were due to variations in the resistance of cells. Amaha and Sakaguchi *(1)* investigated in detail the survival times of the various spore concentrations of aerobic bacilli in neutral phosphate buffer, and have found that the relationships between the survival time and the spore concentration can be shown in the following general equation:

$$\log t = a + b \cdot \log N \qquad (1)$$

where N is the spore number per ml., t is the survival time in minutes, and a and b are the variables depending on strain, temperature, and nature of suspending medium. It was confirmed that the time relationship can not be attributed to variations in resistance of individual spores.

It was the objective of the investigation reported here to examine whether or not the same relationships as shown in equation (1) can be obtained for the anaerobic spores, using Cameron's organism P.A. 3679 (*Clostridium sporogenes*, ATCC 7955) as the test organism.

Townsend, Esty, and Baselt *(29)* and recently Stumbo *(23)*, Stumbo, Murphy, and Cochran *(24)*, Reed, Bohrer, and Cameron *(21)*, Desrosier and Esselen *(13)*, and Reynolds, Kaplan, Spencer, and Lichtenstein *(22)*, have investigated the heat resistance of P.A. 3679 spores. As Stumbo reports in his paper *(25)*, varied heat resistance values are obtained by the investigators, probably because of the varied spore concentrations used by each investigator.

The results of this experiment are believed to serve, to some extent, to standardize the heat resistance values of P.A. 3679 spores to be employed for evaluating thermal process of low-acid canned foods.

EXPERIMENTAL METHODS

Preparation of spore suspensions. A liver broth prepared in the following way was employed for production of spores of P.A. 3679. The chopped beef liver was added to the nutrient broth of pH 7.0 in the amount of 330 g. of liver per liter of broth. The mixture was brought to boiling temperature and allowed to boil slowly for one hour. The boiled material was filtered through filter paper until the broth was brought up to 1000 ml. To the broth there was added 2 g. of glucose and one g. of di-potassium phos-

phate, to adjust it to approximately pH 7.2. *Tubing.* One-half inch of the previously boiled ground beef liver was introduced into each tube and the broth added. The tubes were autoclaved for thirty minutes at fifteen pounds' steam pressure. The final pH after sterilization was about 7.0. The tubes of medium prepared as described above were inoculated with 0.5 ml. of the stock culture of the test organism. The inoculated tubes of media were then heated for 15 minutes in a boiling water bath, to exhaust entrapped air, and subsequently incubated at 30°C. for a period of 30 days. The resulting culture was strained aseptically through a gauze pad to remove liver particles, and then centrifuged. The supernatant broth was decanted off and the residual spore crop suspended again in sterile phosphate buffer of pH 7.0. The suspension was recentrifuged to wash and again the supernatant decanted off and suspended in the phosphate buffer. Then the spore suspension was filtered through a filter paper (No. 2 paper, Tohyo-Roshi Co.) to remove spore clumps. The filtrates (spore suspensions) were then heated for 5 minutes in boiling water to kill the vegetative cells and stored in a refrigerator at $3° \sim 4°C$. Spore concentration was determined by haemocytometer (Thoma).

All experiments in this paper were performed by use of a stock spore suspension which contained 13.2 million spores per ml. and 8.0 million (heat-killed) vegetative cells per ml. It was confirmed that no appreciable variations in heat resistance occurred during the storage for 60 days in the case of P.A. 3679.

Determination of heat resistance. For the thermal death time determinations an aliquot of the stock spore suspension was diluted with sterile neutral M/15 phosphate buffer to give a definite concentration of spores per ml. One ml. of the suspension pipetted into each of the thermal death time tubes; these were of hard glass, 8 mm. in outside diameter, 7 mm. in inside diameter, and about 70 mm. in length. The tubes were then sealed with an oxygen flame, and sets of 20 to 30 tubes were placed in a basket, made of copper screening, for the heat treatment. The basket was completely immersed in a constant temperature oil bath ($\pm 0.15°C$.) equipped with a power stirrer.

A correlation of 3.5 minutes was allowed for the lag period for the contents of the tubes to reach the temperature of the heating bath. At intervals of either 3 or 5 minutes, the baskets were opened and tubes in groups of 4 were removed from the bath and immediately immersed in an ice-water bath. The cooled tubes were wiped clean, flamed, and opened and the tube contents were then aseptically transferred to a freshly exhausted tube of liver broth. The broth was then covered with thioglycolate agar and the tubes were incubated at 30°C. Growth was evidenced by the gas production and the characteristic odor.

RESULTS

Effects of anaerobic maintenance of subculture. The delayed germination or dormancy has been observed by many workers in the case of heated spores of *Clostridium botulinum (9, 14, 17)*. Experiments, therefore, were conducted to investigate the problem with P.A. 3679 spores as well as to establish the length of subcultural period for the thermal-death-time determination.

The spore suspensions with a concentration of one million spores per ml. were prepared and the suspensions were heated at the temperature of 110°C. for various length of time, then subcultured in the following three liver broth media, differing in the methods of maintaining anaerobic condition, (a) liver broth without any stratification, (b) liver broth stratified with non-nutrient agar, (c) liver broth stratified with thioglycolate agar.

During a period of 60 days at 30°C., growth occurrence of each tube was observed daily (Table 1). The observation was continued until 90 days elapsed. But no growth occurred after 30 days of incubation.

The results, as presented in Table 1, indicate that the survival times of P.A. 3679 spores are markedly influenced by the methods of maintaining anaerobic conditions of the same broth. The liver broth stratified with thioglycolate agar gave the maximum survival time, 85 minutes at 110°C., while the other two broths gave the smaller survival times—that is, the liver broth stratified with non-nutrient agar 65 minutes, and the liver broth non-stratified 60 minutes at 110°C., respectively.

These results seem to show that the germination of the heated spores of P.A. 3679 is delayed considerably, corresponding to the length of the time heated, and that when

TABLE 1

Recovery of the heated spores of P. A. 3679 during the subculture by liver-broth with and without stratified agar to maintain anaerobiosis

(Spores suspended in neutral phosphate buffer with a spore concentration of 1×10^6/ml., and heated at 110°C.)

Subculture medium	Time heated	Growth during the subculture at 30°C.						
		3 days	6 days	10 days	14 days	21 days	30 days	60 days
	minutes							
Liver-broth without stratification	40	4[a]	4	4	4	4	4	4
	50	0	3	4	4	4	4	4
	55	0	1	4	4	4	4	4
	60	0	0	2	3	3	3	3
	65	0	0	0	0	0	0	0
	70	0	0	0	0	0	0	0
Liver-broth stratified with non-nutrient agar[b]	40	3[a]	4	4	4	4	4	4
	50	2	4	4	4	4	4	4
	55	0	3	4	4	4	4	4
	60	0	0	3	4	4	4	4
	65	0	0	0	2	4	4	4
	70	0	0	0	0	0	0	0
	75	0	0	0	0	0	0	0
Liver-broth stratified with thioglycolate-agar[c]	45	4[a]	4	4	4	4	4	4
	55	2	4	4	4	4	4	4
	65	0	3	4	4	4	4	4
	70	0	1	3	4	4	4	4
	75	0	0	2	4	4	4	4
	80	0	0	0	2	4	4	4
	85	0	0	0	0	1	3	3
	90	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0

[a] Number of positive tubes. (Four tubes were used for each heating interval.)
[b] Stratified with 2.8% agar.
[c] Stratified with 2.8% agar containing 0.2% sodium-thioglycolate.

the anaerobic conditions of the subculture media are not properly maintained the heat-injured spores fail to recover.

Curran and Evans *(12)* have found that the heated spores of Bacillus are much more exacting in their nutritive requirements than the unheated spores. Amaha *(2, 4)* has found also that the composition of recovery medium has marked influences on the survival times of the spores of *Bacillus natto*, giving the larger survival times when the more suitable nutrients are added to the recovery medium. The results obtained here apparently indicate that in order to obtain the definite values of heat resistance of bacterial spores the conditions of subculture must be preperly maintained with all factors—chemical and physical— suitable for the organism.

From the above results, in all experiments thereafter, the spore suspensions were subcultured by means of the liver broth stratified with thioglycolate agar and the survival times were determined after 30 days' incubation at 30°C.

Effects of spore concentration. The stock spore suspension which contains 13.2 million spores per ml. was diluted with neutral phosphate buffer so as to contain one million spores per ml. Aliquots of the spore suspension thus obtained were diluted to the one-tenth concentration of the original. In this manner, the spore suspensions containing 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, and 1×10^1 spores per ml. were prepared. The spore suspensions were heated at the four temperatures, 105°C., 110°C., 115°C., and 120°C., and the survival times of each spore concentration determined. The results are presented in Table 2. The figures in the brackets in the table show the thermal death times. It is shown that the greater the initial spore concentration (spore

TABLE 2

Relationships between spore concentration and survival time, and the temperature coefficients ($Q_{5°C.}$) of death of P. A. 3679 spores, in neutral phosphate buffer

Spore number per ml.	Survival time, at				$Q_{5°C.}$		
	105°C.	110°C.	115°C.	120°C.	105°~ 110°C.	110°~ 115°C.	115°~ 120°C.
	minutes	*minutes*	*minutes*	*minutes*			
1×10^6	220(240)[d]	85(90)[d]	35(38)[d]	15(17)[d]	2.588	2.428	2.333
1×10^5		72(75)	30(33)			2.400	
1×10^4	150(160)	60(65)	25(27)	11(13)	2.500	2.400	2.272
1×10^3		48(52)					
1×10^2	100(110)	40(45)	17(19)	8(10)	2.500	2.353	2.125
1×10		33(35)					
					Theoretical value: *		
					2.500 → 2.441 → 2.386		

[d] Figures in brackets show the death times.
* Values calculated by equation (4) in the text, assuming the $Q_{5°C.}$ value between 105° and 110°C. to be 2.500.

number per ml.) the longer is the survival times, throughout a wide range of spore concentration from 1×10^1 to 1×10^6 spores per ml.

Now, as is likely the case with aerobic bacilli *(1)*, by plotting the logarithms of the survival times (t) against the logarithms of the spore concentration (N), linear curves are obtained for each of the 4 heating temperatures (Figure 1).

Therefore, it was verified that in the case of P.A. 3679 spores the relationships between spore concentration (N) and survival time (t) could be also expressed by the following formula:

$$\log t = a + b \cdot \log N \qquad (1)$$

For the heating temperature of 105°C. and 110°C., the relationships can be shown respectively as follows:

$$\text{at } 105°C., \log t = 1.434 + 0.084 \cdot \log N$$
$$\text{at } 110°C., \log t = 1.831 + 0.084 \cdot \log N$$

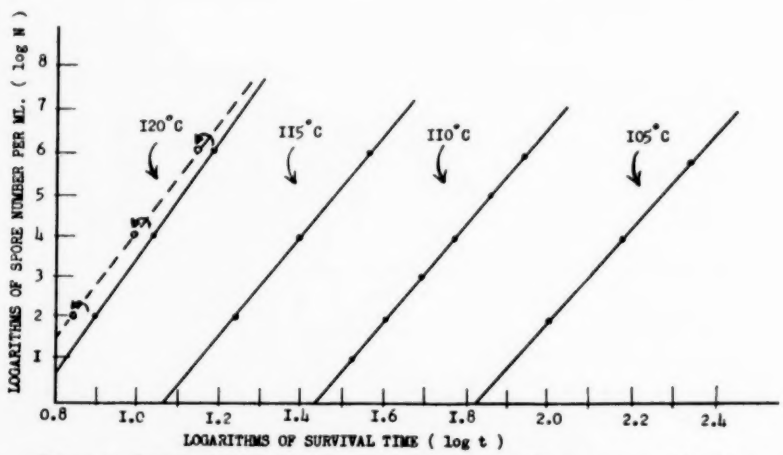

Figure 1. Relationships between spore concentration (N spores per ml.) and survival time (t minutes) of P. A. 3679 spores in neutral phosphate buffer.
(The broken line at 120°C. shows the corrected curve by additional reduction of one minute which was required to reach this temperature.)

Thus, by use of these equations, we can calculate approximately the survival times of any desired spore concentrations at each heating temperature.

The broken line of 120°C. in Figure 1 shows the corrected curve by additional reduction of one minute for temperature rising in the tubes. If the correction for 120°C. was accepted, it can be stated that the 4 "log N ~ log t" curves for the 4 heating temperatures, 105°, 110°, 115°, and 120°C., are approximately parallel to each other. Consequently, in general equation (1), the variable b (which represents the slope of log N ~ log t curve) does not change with temperature, and only the variable a varies with temperatures.

Temperature coefficients and activation energy of death reaction. In the right half of Table 2, the values of temperature coefficient ($Q_{5°C.}$) of death of P.A. 3679 spores were presented for each of the various spore concentrations.

The values were calculated by the following relations:

$$Q_{5°C.} = \frac{k_{T+5}}{k_T} = \frac{t_T}{t_{T+5}}$$

where, k_T, k_{T+5}, and t_T, t_{T+5}, represent the death rate constant and the survival time at the absolute temperatures T and T + 5 respectively.

Comparing the values of $Q_{5°C.}$, it can be seen that the values of $Q_{5°C.}$ of the various spore concentrations approximately coincide with each other in each of the 3 temperature ranges. However, the values of $Q_{5°C.}$ of a spore concentration decrease gradually with the rising of the temperature range.

This fact is not exceptional, because, as Rahn *(20)* pointed out theoretically, the temperature coefficients of a chemical reaction are not constant but gradually decrease with rising temperature. For the sake of comparison, the theoretical changes of the values of $Q_{5°C.}$ were also present at the bottom in Table 2, assuming the $Q_{5°C.}$ value between 105°C. and 110°C. to be 2.500. The results of these analyses, suggest that the death of spores by moist heat proceeds following some rule of physico-chemical nature.

Now, in the same manner as Chick and Martin *(11)* described in their paper on the heat-coagulation of proteins, the activation energy (E) of death reaction of the spores can be evaluated by use of the Arrhenius equation:

$$\frac{d \ln k}{d T} = \frac{E}{R T^2} \qquad (2)$$

Integrating from T_1 to T_2, and converting to common logarithms, equation (2) gives us the form:

$$E = \frac{R T_2 T_1}{T_2 - T_1} \ln \frac{k_2}{k_1} = 4.58 \frac{R T_2 T_1}{T_2 - T_1} \log \frac{k_2}{k_1} \qquad (3)$$

where, k_1 and k_2 are the death rate constant at absolute temperatures T_1 and T_2 respectively, and R is the gas constant (= 2.0 calories).

If $T_2 - T_1 = 5$, $T_1 T_2 = T(T+5)$ and $k_2/k_1 = Q_{5°C.}$, this equation may then be written as,

$$E = 4.58 \frac{R (T+5)}{5} \log Q_{5°C.} \qquad (4)$$

Adopting the $Q_{5°C.}$ values of 1×10^4 spore concentration for the calculation, the following values of E are obtained:

between 105° and 110°C.; $Q_{5°C.} = 2.50$ ..... E = 53,030 calories,
between 110° and 115°C.; $Q_{5°C.} = 2.40$ ..... E = 52,010 calories.

The magnitude of the activation energy as obtained above, supports the theory that the death reaction of spores by heat is the denaturation of a protein molecule in the cells as it is discussed in the previous papers *(3, 4)*.

Thermal-death-time curves for P. A. 3679 spores. By the thermal-death-time data presented in Table 2, the thermal death-time curves for each of the 3 different spore concentrations were constructed (Figure 2).

In this case, both the logarithms of survival times and the logarithms of death times were plotted against temperature, and the thermal-death-time curve was drawn so as to fall above all survival points and below as many death points as possible.

As shown in Figure 2, the 3 thermal-death-time curves for the spore concentration of 1×10^6 per ml., 1×10^4 per ml., and 1×10^2 per mil., were parallel to each other, so

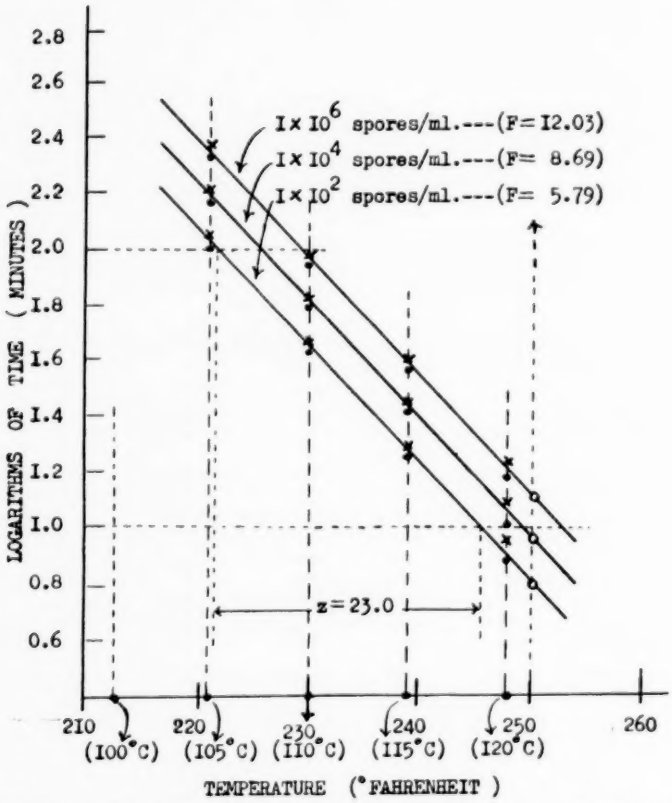

Figure 2. Thermal-death-time curves for P. A. 3679 spores in neutral phosphate buffer.

that z values (the slope of the thermal-death-time curve) were equal to 23°F. for the 3 spore concentrations. However, F values, which represent the number of minutes required to destroy the organism at 250°F., varied markedly with the spore concentrations. From Figure 2, the following F values were obtained for each of the 3 different spore concentrations; $F = 12.03$ for 1×10^6 spores per ml., $F = 8.69$ for 1×10^4 spores per ml., and $F = 5.79$ for 1×10^2 spores per ml.

Then, by plotting the logarithms of F values against the logarithms of spore concentrations, apparently a straight line could be obtained as shown in Figure 3.

Therefore, the relationship between spore concentrations (N spores per ml.) and F values of P.A. 3679 can be also expressed by a linear equation as follows:

$$\log F = 0.6 + 0.082 \cdot \log N$$

This equation gives the F values for every desired spore concentration, when the spores of P.A. 3679 were suspended and heated in neutral phosphate buffer and subcultured by thioglycolate agar stratified liver broth.

DISCUSSION

Thermal-death-time data reported here for P.A. 3679 spores have shown that the relationships between spore concentration (N) and survival time (t) can be shown in the following linear equation:

$$\log t = a + b \cdot \log N \qquad (1)$$

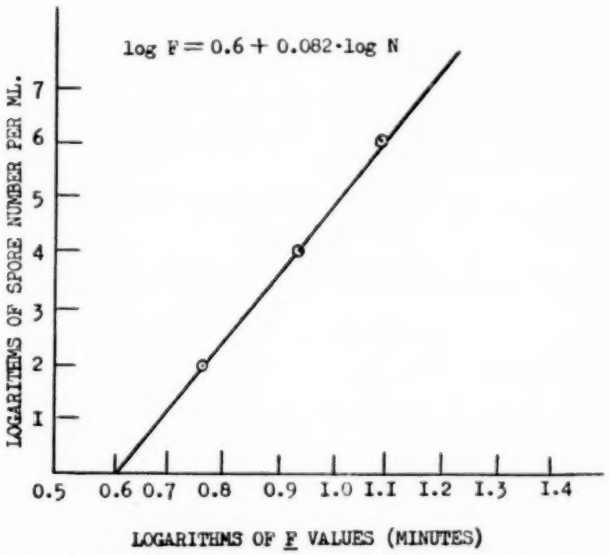

Figure 3. Relationships between spore concentration (N spores per ml.) and F values of P. A. 3679 in neutral phosphate buffer.

Although the data were obtained in the neutral phosphate buffer, these general relationships will probably be substantiated in the food materials, since it has been confirmed in the previous paper *(1)* that when the spores of *Bacillus natto* were suspended and heated in neutral nutrient broth the relationships between spore concentration and survival time could also be expressed linearly by the general equation (1).

The fundamental cause of the linear relationships above cannot be explained as yet. However, it should be pointed out that the similar relationships have been found in the disinfection. In 1908, Watson *(30)*, using the data by Chick *(10)*, showed that the relation of the death rate to the concentration of the disinfectant was best expressed by the following equation:

$$C^n - t_k = \text{a constant (A)} \tag{5}$$

Taking the logarithms of both sides, this equation becomes:

$$\log t_k = \log A - n \cdot \log C \tag{6}$$

where C is the concentration of the disinfectant, n is the concentration exponent, and t_k is the killing time.

The equation (6) represents the linear relation between log t_k and log C, and many workers *(5, 16, 28)* have confirmed the fact that plotting the logarithms of killing time against the logarithms of the disinfectant results in nearly a straight line.

Since C (concentration of disinfectants) can be considered as a relative value to spore number per ml. (N), it can be emphasized here that equation (1) and equation (6) are quite similar.

In the general equation (1) the values of the variable b did not change with temperatures and the values of the variable a only changed with temperatures. Hence, the equations for the 2 different temperatures, T_1 and T_2, may be written in the following equations:

$$\text{at heating temperature } T_1; \quad \log t_1 = a_1 + b \cdot \log N$$
$$\text{at heating temperature } T_2; \quad \log t_2 = a_2 + b \cdot \log N \quad (T_2 > T_1)$$

where t_1 and t_2 are the survival times at temperatures T_1 and T_2 respectively. Therefore,

$$\log t_1 - \log t_2 = a_1 - a_2 \tag{7}$$

and so,

$$\text{temperature coefficient } Q_{T_2\text{-}T_1} = \frac{t_1}{t_2} = 10^{a_1 - a_2} \tag{8}$$

The former relation (7) represents the slope of the thermal-death-time curve at the range of temperature between T_1 and T_2, and the latter relation (8) shows a connection between temperature coefficient and the variable a of the equation (1).

Since the temperature coefficient of a chemical reaction decreases with rising temperature [Rahn *(20)*] and, in fact, $Q_{5°C.}$ values have decreased with increasing temperature as described in Table 2, it is obvious from the relations (7) and (8) that the value $a_1 - a_2$, i.e., the value of slope of the thermal-death-time curve, also decreases gradually with rising temperature.

From the discussion above, it must be noted that the thermal-death-time curves are not exactly straight lines, but very slightly concave downward.

Though the curves can be safely assumed to be straight in the narrow range of temperatures now employed in commercial processes of foods, if the resistance values of an organism at higher temperatures were determined by extrapolation of the thermal-death-time curve of lower temperature ranges, the values obtained would become somewhat smaller than the real resistance values.

In the older methods of evaluation of thermal process *(6, 7, 18)*, the F value of the maximum spore concentration was employed directly for every food material. For instance, for spores of *Clostridium botulinum*, the value $F = 2.45$, which was obtained by Esty and Meyer *(15)* for suspensions containing sixty billions of spores, is customarily employed as the basis for establishing safe commercial processes. Though it would have practically led to safe commercial processes, this procedure of using the maximum resistance value has been critically analyzed by Stumbo *(25, 26)*.

The results obtained in the present paper apparently indicate, as demonstrated in Figure 2, that the F values to be employed in evaluating thermal process should be values properly related to the spore number in foods, in order to make more precise calculations. For this purpose, Stumbo *(26, 27)* has proposed an improved method of process calculation which includes the logarithmic order of death of bacteria.

One of the problems, which should be argued in view of the results of this paper, is the magnitude of the values which represents the initial number of spores of the organism to be destroyed. For the convenience of calculation, Stumbo *(27)* used a concentration of 10 spores per g. of foods

as the initial number of spores to to be destroyed. However, it appears that this number is not well-grounded yet, because of the lack of adequate data concerning the concentration of the heat-resistant bacteria in foods.

There is an urgent need for studies designed to give quantitative data concerning the number of the spores of *Clostridium botulinum*, *Clostridium sporogenes* and other important food spoilage bacteria in a variety of food products.

SUMMARY

The spores of P.A. 3679 *(Clostridium sporogenes)* were suspended and heated in neutral phosphate buffer and then subcultured in liver broth. Marked increases in the survival times of the spores were observed by the presence of stratified thioglycolate agar on the liver broth. The survival times of the various spore concentrations were determined at the 4 heating temperatures, 105°, 110°, 115°, and 120°C. The relationships between spore concentration and survival time of each heating temperature could also be expressed by a general equation, which has been previously obtained on the spores of aerobic bacilli.

Thermal-death-time curves for the 3 different spore concentrations in neutral phosphate buffer were constructed. It was shown that the z values for P.A. 3679 spores are equal to 23°F. for every spore concentration, while F values vary with the spore concentration giving the following values: $F=12.03$ for 1×10^6 spores per ml., $F=8.69$ for 1×10^4 spores per ml. and $F=5.79$ for 1×10^2 spores per ml.

Temperature coefficients of death reaction of P.A. 3679 spores decreased slightly with rising temperature. Consequently, it should be pointed out that the thermal-death-time curve was not essentially a straight line, but was very slightly concave downward.

Acknowledgment

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LITERATURE CITED

1. Amaha, M., and Sakaguchi, K. Studies on the heat resistance of bacterial spores. Part 2. On the relationships between spore concentration and survival time. *J. Agr. Chem. Soc. of Japan*, **25**, 140 (1951).
2. Amaha, M. Studies on the heat resistance of bacterial spores. Part 3. Effects of sugars in the subculture media on the survival times of the spores of aerobic bacilli. *J. Agr. Chem. Soc. of Japan*, **26**, 306 (1952).
3. Amaha, M. Studies on the heat resistance of bacterial spores. Part 4. Kinetics of the death reaction of spores by heat. *J. Agr. Chem. Soc. of Japan*, **26**, 339 (1952).
4. Amaha, M. Studies on the heat resistance of bacterial spores. Part 5. Effects of vitamines and amino acids in the synthetic subculture media on the survival times of aerobic spores. *J. Agr. Chem. Soc. of Japan*, **26**, 420 (1952).
5. Arnold, C. R., Sharf, J., and Levine, M. An equation for the evaluation of the germicidal properties of sodium hydroxide. *J. Bact.*, **34**, 134 (1937).

6. Ball, C. O. Thermal process time for canned food. *Bull. Nat. Res. Council*, **7**, Part 1, No. 37, 76 (1923).

7. Ball, C. O. Mathematical solution of problems in thermal processing of canned food. *Univ. of Calif. Pub. Public Health*, 1, No. 2, 15 (1928).

8. Bullei, *G.* Verbesserte Methode zur Bestimmung des Wertes von chemischen Desinfectionsmitteln. *München med. Wchnschr.*, **51**, 301 (1904).

9. Burke, G. S. The effect of heat on the spores of *Bacillus botulinum:* Its bearing on home canning method. *J. Am. Med. Assoc.*, **72**, 88 (1919).

10. Chick, H. An investigation of the laws of disinfection. *J. Hyg.*, **8**, 92 (1908).

11. Chick, H., and Martin, C. R. On the "heat coagulation" of proteins. *J. Physiol.*, **40**, 404 (1910).

12. Curran, H. R., and Evans, F. R. The importance of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. *J. Bact.*, **34**, 179 (1937).

13. Desrosier, N. W., and Esselen, W. B. Attempts to increase the heat resistance of a putrefactive anaerobe by selection (P.A. 3679). *J. Bact.*, **61**, 541 (1951).

14. Dickson, E. C. Dormancy or delayed germination of spores of *Clostridium botulinum* subjected to heat. *Proc. Soc. Exptl. Biol. and Med.*, **25**, 426 (1928).

15. Esty, J. R., and Meyer, K. F. The heat resistance of spores of *B. botulinus* and allied anaerobes. *J. Infect. Diseases*, **31**, 650 (1922).

16. Gegenbauer, V. Studien uber die Desinfektionswirkung des Sublimates. *Arch. Hyg.*, **90**, 23 (1922).

17. Meyer, K. F., and Lang, O. W. A highly heat resistant sporulating anaerobic bacterium, *Clostridium caloritolerans*, n.sp. *J. Infect. Diseases*, **39**, 321 (1926).

18. Olson, F. C. W., and Stevens, H. P. Thermal processing of canned foods in tin container. Part 2. Nomograms for graphic calculations of thermal processes for non-acid canned foods exhibiting straight-line semi-logarithmic heating curves. *Food Research*, **4**, 1 (1939).

19. Rahn, O. Physiology of Bacteria. Blakiston's Son & Co., Philadelphia, p. 272 (1932).

20. Rahn, O. Physical methods of sterilization of microorganisms. *Bact. Reviews*, **9**, No. 1 (1945).

21. Reed, J. M., Bohrer, C. W., and Cameron, E. J. Spore destruction rate studies on organisms of significance in the processing of canned foods. *Food Research*, **16**, 383 (1951).

22. Reynolds, H., Kaplan, A. M., Spencer, F. B., and Lichtenstein, H. Thermal destruction of Cameron's putrefactive anaerobe 3679 in food substrates. *Food Research*, **17**, 153 (1952).

23. Stumbo, C. R. A technique for studying resistance of bacterial spores to temperature in the higher range. *Food Technol.*, 2, 228 (1948).

24. Stumbo, C. R., Murphy, J. R., and Cochran, J. Nature of thermal death time curves for P.A. 3679 and *Clostridium botulinum*. *Food Technol.*, **4**, 321 (1950).

25. Stumbo, C. R. Thermobacteriology as applied to food processing. *Advances in Food Research*, 2, 47 (1949).

26. Stumbo, C. R. Bacteriological considerations relating to process evaluation. *Food Technol.*, 2, 115 (1948).

27. Stumbo, C. R. Further considerations relating to evaluation of thermal processes for foods. *Food Technol.*, 3, 126 (1949).

28. Tilley, F. W. An experimental study of the relation between concentration of disinfectants and time required for disinfection. *J. Bact.*, **38**, 499 (1939).

29. Townsend, C. T., Esty, J. R., and Baselt, F. C. Heat resistance studies on spores of putrefactive anaerobe in relation to determination of safe processes for canned foods. *Food Research*, **3**, 323 (1938).

30. Watson, H. E. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *J. Hyg.*, **8**, 536 (1908).

THE EFFECT OF PARACHLOROPHENOXYACETIC ACID ON PHYSIOLOGICAL BREAKDOWN OF THE FRUITS OF THE PINEAPPLE (*ANANAS COMOSUS* L. MERR.)

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It was previously reported that pineapple fruits showing physiological breakdown contain less ascorbic acid than normal fruits *(1)*. The results were obtained by analyzing pineapples which had been imported and which had been in transit for a week or ten days with the result that fruits showed breakdown in an advanced degree. Under the circumstances it was not possible to determine whether the loss of ascorbic acid was the cause or the effect of physiological breakdown. However, in subsequent experiments *(2)*, it was discovered that a loss of ascorbic acid occurred in pineapples which had been exposed to low temperatures when the treatment was not severe enough to produce any visible symptoms of physiological breakdown. When it was quite evident that chilling induced a destruction of ascorbic acid, attention was directed toward the possibility of developing treatment which would retard or prevent the loss of ascorbic acid at low temperatures. Mitchell, Ezell, and Wilcox *(3)* reported that spraying snap beans with parachlorophenoxyacetic acid retarded the loss of ascorbic acid following harvest. The authors decided to treat pineapples with this same compound in the hope that the treatment would retard the loss of ascorbic acid and would therefore reduce the amount of physiological breakdown. Growth-modifying substances have been employed for regulating the blooming of pineapple and other tropical plants *(4)* and have been found useful in accelerating the post-harvest ripening of fruits like bananas, apples, and pears *(5)*, but so far as is known to the authors, no one has reported the use of these compounds for reducing the effects of chilling of tropical fruits.

MATERIALS AND METHODS

Concentrated solutions of the spray materials were prepared in Pittsburgh and shipped to Florida to 2 commercial pineapple growers who have cooperated in these experiments for the past 10 years. Parachlorophenoxyacetic acid (PCPA) crystals were dissolved in sufficient "Tween 20" to make the final mixture 1% Tween and varying concentrations of the PCPA as described. In all experiments the sprays were applied to pineapple plants which had been "forced" to bloom by treatment with acetylene during the summer months. Thus the fruits under observation were those which ripened during the fall and winter months and which were most likely to develop physiological breakdown. The solutions were applied to the whole plant by means of a hand sprayer, the rate of application being roughly a quart to 60 plants.

Preliminary experiments were conducted with Abachi pineapples at West Boynton, Florida, during the winter of 1950. Parachlorophenoxyacetic acid was applied at the rate of 200 and 400 p.p.m. from one to 30 days before harvest.

Fruits from plants which had received 400 p.p.m. of PCPA showed less breakdown than the controls but blooming had been irregular so that there were insufficient numbers of fruits in each lot to permit statistical analysis of the results.

Experiments were repeated during the winter of 1951, at which time sprays were applied to Abachi pineapples at West Boynton and to the Natal variety at Sebring. Concentrations consisted of 400 and 800 p.p.m., applied at either 30 or 10 days before harvest. Blooming in the Abachi variety was again irregular and satisfactory numbers of fruits did not mature in time for the prescribed treatment. However, the results obtained were similar to those of the previous year, indicating a beneficial effect of spraying with PCPA.

The Natal pineapples produced uniform blooms so that sufficient numbers of fruits were available for the prescribed treatment. Accordingly four lots were given the same treatment which was applied to the Abachi pineapples; namely, 400 and 800 p.p.m. PCPA, 30 and 10 days before harvest. Two sets of controls were included: one with no treatment and the other receiving Tween 20 only. When the fruits were "mature green," they were harvested and shipped to the University of Pittsburgh by Railway Express. A crate, consisting of roughly 20 fruits, was sampled from each lot except those receiving the treatment 30 days prior to harvest. The latter 2 samples each consisted of two crates, or 40 fruits.

The pineapples usually reached the laboratory in the "turning" stage of maturity. They were permitted to ripen at room temperature before being sampled. When ripe, each fruit was weighed, then cut and observed for degree of physiological breakdown. The data on weight and physiological breakdown were obtained from observation on a total of 163 fruits.

An arbitrary system of classification was set up to indicate the extent of physiological breakdown. The degree was listed as "slight" if no more than 25% of the area of a cut fruit was affected. The fruit was placed in this category even if only one discolored spot, ⅛ inch in diameter, were present. For the most part fruits listed as having slight breakdown could be considered marketable. "Moderate" breakdown was applied to fruits showing 25 to 50% of the cut area discolored. "Severe" breakdown indicated that 50% or more of the area was affected. Fruits with either moderate or severe breakdown were definitely not marketable.

Ten pineapples from each lot were reserved for chemical analyses after observation for breakdown had been completed. These fruits were each sampled individually. They were peeled, cored and macerated in a Waring blendor. The juice was filtered through 2 thicknesses of cheesecloth. Juice volume was determined by means of a graduated cylinder. Total soluble solids were estimated from specific gravity readings which were obtained with a hydrometer. Total acid values represent the result of titrations of aliquots of the juice with standard NaOH, expressing the results as citric acid. The pH determinations were made with a Beckman pH meter. Where average values for pH are presented, the original pH values were first converted to hydrogen ion concentration before averaging. Ascorbic acid values were obtained by titrating aliquots of the juice with sodium 2,6-dichlorobenzenone.

RESULTS

The degree and amounts of physiological breakdown which were observed in the various lots of pineapples are shown in Table 1. Physiological breakdown was found in

TABLE 1

Effect of parachlorophenoxyacetic acid (PCPA) on physiological breakdown in pineapples

Treatment	Number of fruits showing different degrees of breakdown				
	No breakdown	Slight	Moderate	Severe	Total number
No treatment	0	1	7	13	21
1% "Tween 20"	1	3	8	8	20
400 p.p.m. PCPA 10 days before harvest	7	12	1	0	20
800 p.p.m. PCPA 10 days before harvest	12	6	1	0	19
400 p.p.m. PCPA 30 days before harvest	3	14	14	8	39
800 p.p.m. PCPA 30 days before harvest	4	10	18	12	44

varying degrees in all of the pineapples of the untreated controls and in all but one of the fruits which had been treated with Tween 20 only. Spraying the plants with PCPA 10 days before harvest caused a reduction in both amount and severity of physiological breakdown. Lots which were sprayed with 800 p.p.m. of PCPA 10 days before harvest contained 65% sound fruits. Furthermore, even when physiological breakdown appeared in sprayed fruits (treated 10 days before harvest), the disorder was not as severe as in the control lots. When breakdown appeared in the 2 sprayed lots under discussion the degree of disorder was classified as either slight or moderate.

Treating pineapple plants with PCPA 30 days before harvest was not nearly as satisfactory as was the treatment involving the shorter period. This was true for both the applications of 400 and 800 p.p.m. A few more sound fruits were found in these lots than in the controls but the differences were not great and the amount of control of breakdown cannot be considered economically important. Additional disadvantages of the longer interval between application of the spray and harvesting of the fruits will be apparent from consideration of some of the other observations. These are presented in Table 2.

TABLE 2

Effect of parachlorophenoxyacetic acid (PCPA) on weight, volume of juice, and juice constituents of the pineapple

Treatment	Average of 10 individual fruits					
	Weight	Juice vol.	Total soluble solids	Total acid	pH	Ascorbic acid
	g.	*ml.*	*%*	*g. per 100 ml.*		*mg. per 100 ml.*
No treatment	591	150	10.6	1.02	3.42	14.3
1% "Tween 20"	578	138	10.6	1.10	3.39	25.4
400 p.p.m. PCPA 10 days before harvest	600	144	11.1	1.15	3.39	38.7
800 p.p.m. PCPA 10 days before harvest	625	153	11.0	1.19	3.44	40.6
400 p.p.m. PCPA 30 days before harvest	474	98	10.4	1.17	3.49	30.1
800 p.p.m. PCPA 30 days before harvest	475	106	10.1	1.03	3.51	16.9

Treatment with PCPA 30 days before harvest resulted in significant reduction in the weight of the pineapples. Average weights of fruits in these lots were 474 g. for those receiving 400 p.p.m. and 475 for the 800 p.p.m. application. The average weights of the other lots ranged from 578 to 625 g. Differences between the weight of the controls and those receiving PCPA 10 days before harvest were not significant. The lower juice volumes in the 30-day lots are merely reflections of the reduced weights of the fruits.

No significant differences were observed between treated and control lots in regard to total soluble solids, total acids or pH. In like manner differences between lots receiving no treatment and those receiving Tween 20 only were not significant.

Greatest contrast between treated and control lots was observed in ascorbic acid content. The fruits which received 400 p.p.m. 10 days before harvest contained 38.7 mg. of ascorbic acid per 100 ml. of juice and those receiving 800 p.p.m. at the same time contained 40.7 mg. The ascorbic acid content of the controls and those receiving Tween 20 only was 14.3 and 25.4 mg. per 100 ml., respectively. The differences between treated and control lots are statistically significant. Although there was more ascorbic acid in the lots receiving Tween only than in those receiving no treatment, these differences are not significant.

Ascorbic acid in the other lots, i.e., those which were sprayed 30 days before harvest, was slightly higher than in the controls but the differences were not significant.

Two other effects of these sprays are observed. Crowns of treated fruits appeared smaller than those of the controls. Also approximately 10% of fruits receiving 800 p.p.m. PCPA lost their crowns either during transit or following their arrival at the laboratory. This was true for this concentration irrespective of the time of the application of the spray. A concentration of 400 p.p.m. of PCPA showed no effect on crowns other than to reduce their size.

DISCUSSION

Results of these experiments show rather clearly that physiological breakdown of pineapples can be considerably reduced by properly-timed spraying of the plants with a solution of 800 p.p.m. of parachlorophenoxyacetic acid. Treated lots contained few fruits showing breakdown and when it was present, the disorder was less pronounced.

Classification of disordered pineapples into slight, moderate, and severe stages is entirely arbitrary and therefore subject to a certain amount of error. A better criterion of the effectiveness of the spray may be obtained by determining the ascorbic acid content of the fruits. It has been pointed out that chilling of pineapples results in a loss of ascorbic acid and that in severe cases of breakdown most of this vitamin may have been lost. It will be observed that in these experiments the lots which showed the largest number of sound fruits likewise had the highest content of ascorbic acid. This represents a more quantitative means of estimating the effectiveness of the spray.

The loss of tops in 10% of the fruits receiving the higher concentration of PCPA emphasizes the need for additional studies before making general recommendations for commercial application of the compound. It is possible that a slight change can be made in the formulation which will eliminate the slight, deleterious effect on tops without affecting effectiveness of the spray.

In the present tests the advantages of the treatment outweighed the disadvantages. The incidence of breakdown in winter-ripened pineapples may be very high in certain varieties and has been known to affect the whole crop.

In the era when Florida-grown pineapples were all harvested during the summer months, physiological breakdown was observed only in fruits following extended periods of storage or transportation, and the disorder occurred rather infrequently. Under the conditions of the present practice of forcing the bloom so that pineapples ripen during the winter, physiological breakdown has become a much more serious problem. Exposure of mature green fruits in the field to temperatures between 32 and 40°F. for a period of 48 hours may induce physiological breakdown in the entire crop.

Like many other "out-of-season" fruits, winter-ripened pineapples command higher prices and any treatment which maintains the fruits in a marketable condition would be welcomed by representatives of this rather specialized industry.

SUMMARY

Natal pineapples on a commercial plantation in Sebring, Florida, were sprayed with parachlorophenoxyacetic acid at the rate of 400 and 800 p.p.m. Applications were made on one group of plants 30 days before harvest and on another 10 days before harvest.

When applied 10 days before harvest both concentrations of parachlorophenoxyacetic acid reduced the amount of physiological breakdown in the fruits, the higher concentration being the more effective. The percentage of sound fruits resulting from treatments applied 10 days before harvest

was as follows: Control, 0%; 400 p.p.m., 35%; 800 p.p.m., 63%. Sprayed fruits contained significantly higher quantities of ascorbic acid than the control pineapples.

Application of the spray 30 days before harvest produced no significant reduction of physiological breakdown but resulted in a slight reduction in size of fruits.

The crowns of the treated fruits were slightly smaller than those of the controls. Roughly 10% of the fruits receiving 800 p.p.m. parachlorophenoxyacetic acid had lost their crowns upon arrival at the laboratory.

Total soluble solids, total acids, pH, and flavor of the juice were not affected by any of the treatments.

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LITERATURE CITED

1. Miller, Erston V. Physiological studies of the fruits of the pineapple (*Ananas comosus* [L.] Merr.) with special reference to physiological breakdown. *Plant Physiol.*, **26** (1), 66-75 (1951).
2. Miller, Erston V., and Heilman, Alan S. Ascorbic acid and physiological breakdown in the fruits of the pineapple (*Ananas comosus* [L.] Merr.). *Science*, **116**, 505-506 (1952).
3. Mitchell, John W., Ezell, Boyce D., and Wilcox, Marguerite S. Effect of p-chlorophenoxyacetic acid on the vitamin C content of snap beans following harvest. *Science*, **109** (2826), 202-203 (1949).
4. Mitchell, John W., and Marth, Paul C. *Growth Regulators for Garden, Field and Orchard.* 1947, The University of Chicago Press, Chicago, Illinois.
5. *Plant Growth Substances.* Edited by Folke Skoog, 1951, University of Wisconsin Press.

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